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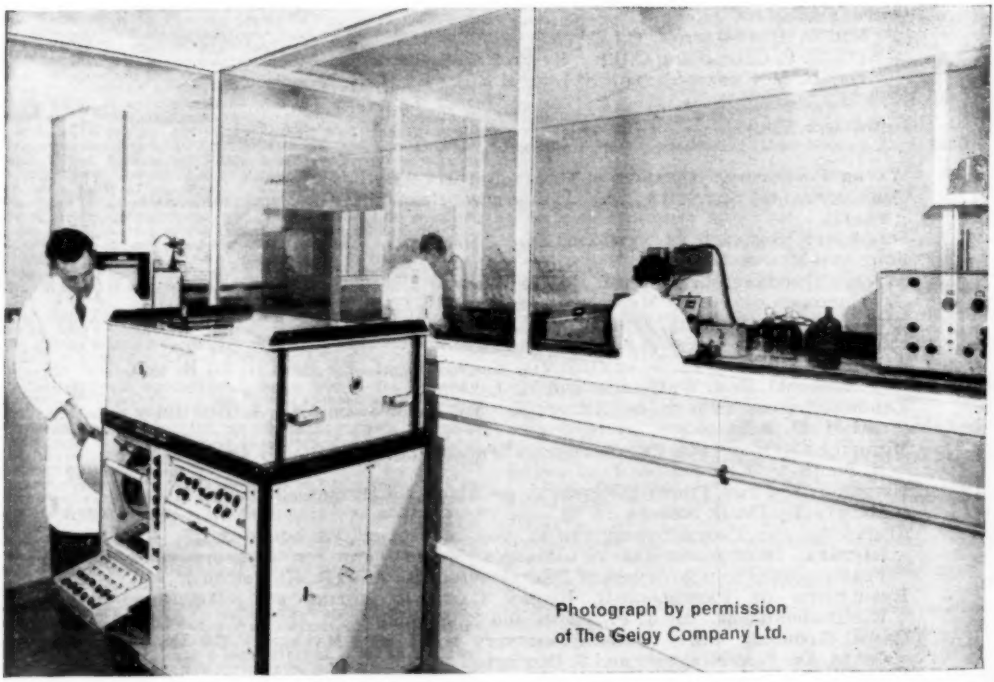

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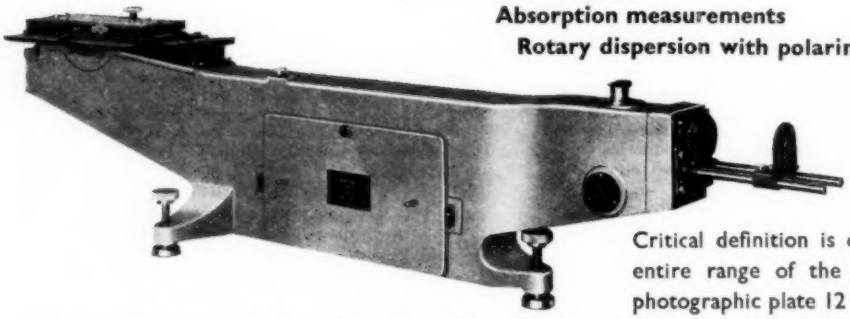

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PROGRESS IN INDUSTRY

A PAPER which was read by Mr. M. Zvegintzov before Section F (Economics) at the Norwich meeting of the British Association on September 1 examined some of the accusations which have been levelled against British firms—and even whole industries—as unprogressive or backward or obsolete, in contrast to those of other countries. The paper also advanced some constructive suggestions for remedying this position in so far as it can be substantiated. A recent book by Michael Shanks, for example, roundly condemns the British economy as a stagnant society; and, although he may have picked out industries which are scarcely representative in regard to the proportion of employees with technical qualifications—in the example quoted it was 0·6 per cent—some of his more general charges have considerable substance. Mr. Shanks is by no means the first to point out that Britain should, by and large, be relying on brain-power for exports, and exporting the products which require scientific and technical skills, or even exporting her own trained man-power, rather than exporting the products of bulk production. Reliance on the amateur and distrust of the specialist are admitted weaknesses; and that in too many British firms promotion comes by nepotism rather than merit is unlikely to be challenged within the reservations which Mr. Shanks rightly makes, even by those indisposed to accept his view that our class system is mainly responsible for all these weaknesses.

Mr. Zvegintzov begins by pointing out that the man-power outlook in Britain is extremely tight, but he anticipates a useful increase in the productive age-groups by 1985, when the population is expected to reach some 56 million. What he stresses more particularly, however, is the need to take steps to re-deploy continuously for new demands the limited available man-power. This he regards as a *sine qua non* to avoid a halt in the rise of the standard of living. The expansion of world trade, the industrialization of undeveloped countries and rising standards there and in the developed communities, all put the emphasis on a change in the character of world trade, and this assumption is more or less implicit in the recent report which attempted to forecast the long-term demand for scientific man-power.

With this capacity for change must go flexibility and mobility. Mr. Zvegintzov is ruthless about real backwardness and unadaptability and looks unequivocably to the elimination of such firms by the process of economic competition. To attempt to preserve them by protective measures or restrictive practices would be disastrous and place an unjustified strain on the limited resources of man-power in Britain; the critical problem is really how to translate the valuable craft tradition and accumulated experience which characterize a substantial proportion of the medium size or smaller firms, which constitute a significant proportion of British industry, into a modern scientific idiom. The aim, as he sees it, should be to devise and supply to the craftsman the most modern and delicate tools which will enhance his craft, and this in turn involves further capital investment with saving in labour, and, even more important, educating the craftsman to demand these additions to his craft.

Acceptance of this challenge by scientist and technologist as well as technician or craftsman and industrialist could well falsify some of the estimates made by the Committee on Scientific Manpower, although the question of technicians is still to be examined by it. Mr. Zvegintzov rightly points out that success here would go far to enable us to meet overseas competition, and that with a high craft tradition, using delicate instruments and high-precision tools for detecting, initiating and maintaining adjustments, very high standards as well as flexibility could be achieved without recourse to the further research and high expense which would be necessary for automatic production with low-grade labour. Moreover, he visualizes the possibility of easy transfer of the habit of mind and inherited skill, which characterize craftsmanship, to other and perhaps new requirements as in the design and construction of scientific instruments.

Perhaps the most important part of Mr. Zvegintzov's paper, however, is that in which he discusses the difficulties that attend innovation in long-established and well-developed industries, and particularly in deciding where further investigation or development is likely to be most worth while. It is essentially a question of how much research and where, and Mr. Zvegintzov lays particular stress on the importance of effective communication between science and technology on one side, and finance and commerce on the other. He visualizes, for example, a detailed cost breakdown analysis, possibly with comparisons from other countries, to bring out the features where the incidence of cost is considerable, followed by comparisons of this breakdown in a group of firms; and finally a joint study by technologists and cost-accountants to assess whether any scientific or technical innovation, or even a complete re-orientation of operations, could remove or ease this incidence of cost.

In all this, close co-operation is imperative at working-level, alike between firms and between management and accountancy and the technologist, particularly if budgetary control techniques are not to confuse ends and means and impose rigid procedures which ignore circumstances and stifle initiative. This kind of analysis, Mr. Zvegintzov points out, has not been tried, though departments of economics or industrial management at universities in certain industrial areas would appear to be well placed to undertake it. This is possibly due to the absence of finance for this type of experimental economics, and, if so, it should be easily remedied if

the Chancellor of the Exchequer's new structure is to stimulate expansion and development in industry in Britain.

It is worth noting that something of this is foreshadowed in a paper which Mr. G. S. J. White contributed in January 1960 to the *Journal of the Society of Dyers and Colourists*. Limiting himself specifically to the development of dyestuffs for textiles and looking especially at the needs of the consumer, Mr. White sought to assess the magnitude of the research effort which it is worth while expending on the discovery and development of new dyestuffs. It is exactly this type of critical and realistic appraisal that is all-important if Britain's limited resources of trained man-power are to be deployed where they offer the greatest prospect of benefit to the national economy, and it is at this point that Mr. Zvegintzov's analysis links up with the importance of effective management.

It is indeed to the limitations of management, particularly in smaller firms, that Mr. Zvegintzov attributes the lack of receptivity to new ideas, and he does not seem optimistic as to the possibility of overcoming such resistance otherwise than by the use of consultants. This, in turn, he admits, depends on the initiative of managements if effective use is to be made of such consultants; but he sees ground for hope in the expansion of the colleges of technology and of the scientific and technological faculties at the universities. Apart from its direct educational influence, this expansion offers a potential source of consultants and contacts between industry and the frontiers of science and technology, which should create a much more favourable climate of opinion in industry; and for this reason alone every effort should be made to encourage the movement of staff between industry and the universities and colleges of technology.

How far the regional offices of the Department of Scientific and Industrial Research could assist in this process may be open to debate; but, from the point of view of information service alone, there seems sound sense in Mr. Zvegintzov's plea that these regional offices should be strengthened and staffed with able men. Moreover, there can be no doubt that the Research Associations could function very effectively here, and that their educational function, both from the point of view of information and as a source of trained staff, has been under-estimated or overlooked in the past. The Robbins Committee could well give more than a passing glance at the position and functions of the Research Associations in the organization for higher education in Britain; but meanwhile the Council for Scientific and Industrial Research should examine the contribution which the Associations could make in such ways to industrial efficiency and development.

Mr. Zvegintzov is indeed rather more optimistic as regards the future of the smaller firms, because he believes a new generation of management is coming into office which is much more alive to scientific and technological opportunities. This is the more important for, as he also contends, the rapid progress of technological co-operation and even integration in Europe may present a more important feature of the Common Market than ordinary commercial competition. Here the influence of the Research Associations may be beneficial, though it is likely to be weakest in the traditional industries where it is needed most. Incidentally, he comments here on the need for more research in the human sciences, where industry still relies far too much on guess-work; but he suggests that for the smaller and more specialized firms a powerful stimulus to co-operation could come from their suppliers of materials or equipment, and from the users of some of their products. Most of these firms are bigger and with wider contacts both at home and abroad, and thus may be better placed to know what is new and promising.

Whether in fact such firms may be willing to help with expert advice is a matter of opinion, and Mr. Zvegintzov could be too optimistic on this point, though he suggests the possibility of joint studies under some co-operative trade association. Here the Common Market could help in providing a large pool of experience, and Mr. Zvegintzov in fact suggests that entry into the Common Market could provide the biggest single step for stimulating the unprogressive; though if, in fact, the operative factors are financial, the policy initiated by the Chancellor of the Exchequer at the end of July should be directed to that end. Nevertheless, where the process acquires also much qualitative judgment, involving identification of needs, assessment—qualitatively and quantitatively—of advantages, and the establishment of priorities, it is more than a little doubtful how far the Exchequer is likely to be of direct assistance.

The changes recently recommended by the Plowden Committee should go some way, however, to facilitate the kind of outlook that is required, and some of Mr. Zvegintzov's concluding words are strikingly in line with that report. Continuous progress, he believes, can only come through enterprise and boldness, which are only likely when there is confidence in the future and assurance of continuity. The general climate of opinion is thus psychologically very important, and for this reason alone he condemns the current 'stop–start' policy as extremely dangerous. Furthermore, the inexorable consequences of entering into scientific commitments cannot be ignored, and to insist that some co-ordination is needed between official scientific and economic organization is not to advocate rigid planning on the Russian model.

Mr. Zvegintzov himself leans to something on the lines of the French system of the Commissariat au Plans, attached to the Prime Minister's Office; but he insists that above all development must come from within industry itself. There is no substitute for efficient self-management, and probably the wisest national policy for Britain is one that provides definite encouragement to the enterprising and efficient, removing obstacles and providing positive advice and even help, rather than one that attempts to stimulate the backward or unprogressive.

Much of Mr. Zvegintzov's argument, it is true, can be traced in Sir Harry Pilkington's Fawley Lecture of three years ago, "Facing the Future in Industry". Sir Harry made the vital points about effective leadership, the choice of priorities, particularly in regard to the direction of research, and the real co-ordination of research, technical development and production; but it is hard to point to substantial progress in many sectors of industry since that Lecture was given. Nor does Mr. Zvegintzov himself really face some of the issues that can arise in such an industry as shipbuilding or the machine tool industry, if the industry itself really fails for internal reasons to meet the challenge. Is the Government to stand idly by if employers fail to seize their opportunities and make the necessary adjustments, or if trade unions cling to obsolete and restrictive practices that prevent full advantage being taken of technical advance?

It would be foolish to pretend that Sir Harry Pilkington, Mr. Zvegintzov or Mr. Shanks has provided the answer to these problems, nor is it probable that they would claim to have done so. They have indicated certain aspects of the problem which call imperatively for further and urgent examination and for some clear and constructive thinking. There are matters which the Minister for Science as well as the Chancellor of the Exchequer and their advisers should be examining; and there are others to which the Minister of Education should be giving attention. But the major challenge is to industry itself, and to its co-operative organizations, both of employers and trade unions. The situation is far too serious for either management or labour to rest content with putting the blame on others even for admitted mistakes. The need for efficient management is once again overwhelmingly demonstrated, with all that that implies regarding training and selection for management. No less clearly, however, is demonstrated the need for thinking about production in terms of the techniques and possibilities of to-day, and not in terms of practices of a previous century; and in that the trade unions have at least as large a responsibility as that of management.

The primary emphasis, moreover, must be on flexibility, imagination and information. On information, because it is only the free dissemination of information that prevents the duplication of effort which is so wasteful of resources—material and manpower—and provides the basis for sound judgment as to the objectives most worth while in research or in development. That reason justifies the Committee of Public Accounts in pressing for more informative accounts from the Atomic Energy Authority, and the relevant passage in the Committee's Third Report for the Session 1960–61* should not pass unnoticed. The Committee fully appreciated that it is necessary on security grounds to restrict the information provided by the Authority's published accounts; but although the Public Accounts Committee was concerned in this instance more particularly with the control of recoverable expenditure relating to reactor research and development, and the manufacture and sale of fuel elements, it is clear that it is not satisfied that sufficient information is disclosed as to the Authority's expenditure on research and its distribution.

Imagination is necessary, because on this factor depends very considerably the ability of Great Britain to exploit some of the possibilities of new techniques in automation and the electronic computer. Some of these possibilities were indicated by Dr. B. V. Bowden in his evening discourse, "The Impact of Automation", before the British Association at Norwich, when he hinted at possibilities of their use by the Exchequer in economic controls. The importance of imagination both in the use of such techniques and in training a generation of scientists competent to use them was particularly well stressed by Prof. R. Courant in an address last year at the closing session of an international symposium arranged by the International Computation Centre in Rome and which has now been published in *Impact*.

Flexibility is also essential, because of the demands for adjustment and a new outlook which it makes at all levels. There is the demand on education to provide men and women with minds alert to the new possibilities and equipped to use the new opportunities. There is the demand on management with which Mr. Zvegintzov dealt, which involves not merely facing present-day problems of development but also foreseeing the human and technical problems involved in expansion, in automation and in redundancy, and in the recruitment of managers competent to deal with these problems in the future. There is the demand made on manager and trade unionist alike to face these problems as they arise, and to adjust practices to new needs, developing habits of co-operation rather than restriction, and preparing for change rather than shrinking from it or ignoring it. Solutions to these interlocked problems will not be found in isolation, nor can the necessary policies be implemented without mutual understanding and co-operation, and the task of creating in industry and in Britain generally the appropriate climate of opinion is one to which all possible efforts should already be directed.

* Third Report from the Committee of Public Accounts, Session 1960–61. Pp. 40. (London: H.M.S.O., 1961.) 2*s*. net.

COSMIC RADIO WAVES

Cosmic Radio Waves

By I. S. Shklovsky. Translated by Richard B. Rodman and Carlos M. Varsavsky. Pp. xvi+444. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1960.) 65*s*. net.

THOSE of us who were fortunate enough to receive a copy of Shklovsky's book when it was first published in Moscow in 1956 realized that a significant contribution had been made to radio astronomy by this Russian scientist. Now the value of the book, and the understanding of Shklovsky's personal contribution, has been greatly increased by the appearance of this English translation. The author has himself revised the work to bring it up to date so far as the contributions to radio astronomy which

were presented at the Paris Symposium in 1958. Even so, in view of the tremendous growth of the subject and the productive research of the world's major radio telescopes since that date, it might be expected that the book would already be too old to be useful. In fact, the book makes vivid reading and will be an inspiration to all concerned with the problem of radio waves from the cosmos.

The main reason for this timeless quality of the work is to be found in the great theoretical contribution which Shklovsky has himself made to radio astronomy. About ten years ago radio astronomers in the West were under the influence of the radio star idea as an explanation of the origin of the radio waves from the local galaxy, by which a distribution of radio sources somewhat similar to that of the common stars was believed to be the probable origin of the galactic radio emissions. Shklovsky held that this view was untenable. He believed that the main generally distributed radio waves from the galaxy had two separate origins, first, a 'flat' component closely concentrated in the plane of the Milky Way arising as an emission with thermal characteristics from the free–free transitions in the ionized hydrogen gas in the plane of the galaxy; second a 'corona' or 'halo' component, surrounding and enclosing the galactic system of stars and arising as synchrotron radiation from relativistic electrons moving in the interstellar magnetic field. He believed that a few of the intense discrete radio sources of emission were supernovæ remnants lying in the plane of the galaxy (as exemplified by the already well-known case of the Crab nebula in Taurus), and that the majority were extragalactic.

At first Shklovsky's views received little attention, but during the past few years there has been clear observational proof that they provide a reasonable explanation of the emission both from the local galaxy and from the individual extragalactic nebulæ. Shklovsky's explanation in this book of the analytical procedure by which he arrived at these conclusions from a study of the spectra and isophotes of the radio emission made by radio telescopes which confused the issue by their poor resolving power is a masterly example of scientific procedure which should be studied by all young research workers.

Another long section of the book which deals with the emission of light and radio waves from the supernovæ remnants still further exemplifies the fruitfulness of the conceptions of Shklovsky and his Russian colleagues. The discovery of the polarization of the light from the Crab nebula, and (since the book was revised) of the polarization of centimetre radio waves from the nebula, provides firm observational support for these ideas that the emission arises as synchrotron radiation from the motion of high-speed electrons in the magnetic field of the nebula. In due course these electrons move away from the nebula to form the electrons in the corona of the galaxy and to become, with other energetic particles resulting from the explosion, the primary particles of cosmic rays.

The scope of the book is specifically limited to the problem of the cosmic radio waves of galactic or extragalactic origin. The radio or radar astronomical aspects of the subject concerned with the solar system are excluded. For all who are interested in the cosmos this book makes exciting reading, while for astronomers of all kinds and for those physicists who are concerned with the problem of the origin of cosmic rays the book will be an invaluable acquisition.

Bernard Lovell

THÉODORE SCHWANN

Lettres de Théodore Schwann (1810–1882)
Éditées et annotées par Prof. Marcel Florkin. Pp. 274. (Liège: Société Royal des Sciences de Liège, Université de Liège, 1961.) 250 francs.

THE 'cell-theory' is the story of the recognition of the cell as the essential unit in the structure and function of the body. The first suggestion of this coming change occurred in the work, *Die Zeugung*, which Lorenz Oken produced in 1805, but the important dates are 1838 and 1839, when Matthias Jacob Schleiden (1804–81) and Theodore Schwann (1810–82) enunciated in clear form the new ideas.

In the volume reviewed here a distinguished Liège professor and medical historian shows how the second of these two men, who was born at Neuss-am-Rhein, apparently failed to justify the high hopes of his earlier years. He had shown great promise when he was working in the laboratory of Prof. Johannes Müller in Berlin, but in 1839 he became a teacher in Louvain and afterwards in Liège; and in Belgium he published nothing but a few unimportant treatises and a brochure accompanying the apparatus which enabled one to exist in an irrespirable medium; it was shown in the Universal Exhibition in Paris in 1878.

F. G. J. Henle (1809–85) said that Schwann, during his five productive years, did enough for science to justify the entire lifetime of a great man. He also noted that Schwann had been forced to lose much time studying systematic human anatomy when his real forte was physiology, and, moreover, when the disputes about general anatomy were repugnant to his sensitive nature.

At Louvain and at Liège the Roman Catholic Church was not concerned with the matter, for only at the end of his life did Schwann become a devotee to the faith, and he was unhampered in both his ideology and his religious experience.

In 1842 at Louvain he began an intimate diary or *Tagebuch innerer Ereignisse*, part of which was published in 1936. An unpublished part, of exceptional autobiographical interest, was produced by Florkin in 1951, and there is also a series of treatises by Schwann relating to his inner life.

Three periods can be distinguished in his story, namely, an ethical one, during which he strove for knowledge and the perfecting of self, a scientific one, which was rational and Promethean, and a mystical or metaphysical one, all three being characterized by intellectual eagerness. Schwann's contemporaries and the generations separating us from him knew only his scientific output, considering it an intellectual oasis in the general drabness of a life devoid of eminence. They were unaware that behind the decorous existence of the famous and conscientious but unproductive faculty professor was being carried on an unceasing intellectual struggle.

Florkin enlarges on these three periods. In the first he has much to recall about one Wilhelm Smets; in the second he recaptures the liberal Berlin atmosphere dominated by the powerful personality of Johannes Müller, and Schwann's attempts to make biology a province of physics. In an appendix to his *exposé* of his cell-theory, Schwann disclosed a philosophy which rejected vitalism and sought the final cause of the universe in the actual properties of atoms. He affirmed the physico-chemical nature of life, this affirmation being his crowning achievement as a

scientist, taking him away from scientific inquiry and orienting him towards metaphysics. It was at this period in 1838, in the course of an attack of conscience, that he renounced rationalism and the scientific method in an attempt to know his Creator and thereby truth.

Florkin forecasts that Schwann's personality will certainly stimulate various works, not only by historians of science but also by psychologists given to the 'characterology' of the scientific investigator and to that of the meanderings of the spirit of man.

Besides renewing biology by his discoveries and scientific theories, Schwann's philosophic and religious pre-occupations, and the transfiguration of science into religious emotion which he sought in his theories, the intensity of his inner life and his long resistance to orthodoxy outline a life at once sad and serene, untiringly devoted to the most exalted and moving aspirations of the human soul. As Edmond Scherer said of another great and lonely figure, Frédéric Amiel, Schwann was "de ceux qu'a touché de son aile l'ange des visions ineffables et des divines tristesses".

KENNETH J. FRANKLIN

WEALTH OF THE FOREST

Les Plantes Utiles du Gabon

Essai d'Inventaire et de Concordance des noms Vernaculaires et Scientifiques des Plantes Spontanées et Introduites. Description des Espèces, Propriétés, Utilisations Économiques, Ethnographiques et Artistiques. Par André Raponda-Walker et Roger Sillans. (Encyclopédie Biologique, Tome 56.) Pp. x+614+53 planches. (Paris: Éditions Paul Lechevalier, 1961.) 250 NF.

TO us who depend increasingly on 'supermarket' and slot-machine, one of the most interesting aspects of life in less sophisticated lands is the wide use made of local plants to supply daily needs. Nearly every plant seems to have its use; this one as a cure for fever, the next providing fibre for fish-nets, a third giving wood for musical instruments. Of interest, therefore, is a new book describing useful plants of that western part of equatorial Africa known as Gabon. Dr. Albert Schweitzer's reference to this coast as the edge of the primeval forest comes to mind, and, although the vegetation of the coastal and riverine areas is now well known and is much altered by man's activities, the vast area of the interior, mainly forest, remains inadequately recorded.

In a pleasant foreword by Prof. Trochain, the book is described as the joint product of a priest-botanist-ethnologist and a botanist-draughtsman-artist. During thirty years of missionary work in that country, the senior author, the Abbé Raponda-Walker, amassed the information on plants and their local names and uses which forms the bulk of the text. The notes being placed in the hands of R. Sillans, he attempted, as is explained, to bring the material to life, to depict the vegetation of the region as known to the inhabitants and as it enters into their daily life.

The result is a book which is full of information for both botanist and ethnologist. It opens with short introductory chapters on the history of botanical exploration in Gabon, on the climate and types of vegetation, and the vegetable products entering trade. In the main part of the book, the plants which are put to use are described in turn, the genera being arranged alphabetically in their families. For each species is given a short description, a note of its use in native custom, and a list of vernacular names by which the plant is known among various of the 27 tribes in the country. The artist provides 52 full-page botanical drawings of important species. In addition, the text is enlivened by figures and illustrative sketches. If the writer tells us that wood of *Alstonia* is used for ceremonial masks, the artist employs his pen to show the masks worn by some tribal society in their fetish rituals. The book is well indexed, and nearly 8,000 entries in the index of vernacular names are a measure of the Abbé Raponda-Walker's diligent observation.

Many plants described here are common also to forest country of West Africa to the north-west, and it is possible to compare the uses of them in Gabon with those so well described earlier by Dalziel, or in Irvine's *Woody Plants of Ghana* (see p. 695 of this issue). Others again are peculiar to the region of Gabon, ranging in economic importance from the great okoumé tree, *Aucoumea klaineana*, the timber of which constitutes the greatest wealth of Gabon, to so small and nasty a plant as *Ataenidia* of the arrowroot family, the leaves of which, thrown into the cooking-pot, cause discord in the household or village. Ferns are among the plants of which use is made; and an unusual record is the weaving of the black shining rhizomorphs of a fungus into ornamental cords to wear on the body. An appendix gives the vernacular names of recognized cultivars of some staple crops, including banana, cassava, and sugar-cane.

The useful plants of Gabon, as here presented, include many introduced plants. Some, like banana and maize, have long been adopted by the Gabonese and have become part of their life. Others have not penetrated farther than the trial-ground or mission-garden, and, although mention of this is made, their appearance at all in the main text as useful plants of Gabon may well be questioned. To find included the Judas tree, *Cercis siliquatrum*, a Mediterranean plant ill-suited to the humid tropics, is as unexpected as it may be misleading. But this, and the mis-spelling of some botanical names, are minor blemishes in a work which contains much of value and of charm.

T. A. RUSSELL

FORMAL LOGIC IN RETROSPECT

A History of Formal Logic

By Dr. I. M. Bochénski. Translated and edited by Ivo Thomas. Pp. xxii+567. (Notre Dame, Indiana: University of Notre Dame Press, 1961.) 20 dollars.

IN this monumental work, Prof. Bochénski presents us with the results of some forty years of research. Scholars are already aware of the merits of the German edition, published in 1956, especially the accuracy of the transcripts from Aristotelian texts. Now we are enabled to have the outcome of this 'labour of love' in English, thanks to the University of Notre Dame Press, and a competent translator. In the event, these pages are very much more than a source-book. They will be consulted by logicians and historians for many a generation maybe, before anything comparable appears again. The decimal system of entries—both extracts and commentaries—of the original is maintained with trifling exceptions, which makes reference easy within the volume itself, and to the German version.

XUM

An introduction to the present edition has been added, which has as its main object the slightly unusual one of augmenting the very brief (and perhaps inadequate) treatment of Abelard in the original. It is good to have this, though one would have thought that a more appropriate place could have been found for it. But we are left in no doubt that formal logic was well away in its scholastic form by the twelfth century.

In his own introduction, the author says plainly what he conceives his task to be, and how he intends to carry it out. Logic did not develop continuously; rather it started at various places and at various times almost independently. But, characteristically, men soon found that they were grappling with much the same essential problems, approaching them by different paths. This concept sets the pattern for the whole book. Thus we have ancient logic, scholastic logic, mathematical logic, and Indian logic. The somewhat arid period of 'classical' logic is briefly reviewed. Before discussing these sections in greater detail, it is clear that two figures stand out in bold relief, Aristotle and Frege, the rest grouped around them with the symmetry of an artist. It is almost as if Prof. Bochénski had painted a new version of Raphael's great picture, adapting the stance of the figures to subsequent history as the grand drama of the pursuit of reason unfolds.

Ancient logic (Part 2) shows the author at his best, and text is superb. Here is the essence of the esoteric syllogism, displayed both in its intellectual power and its æsthetic appeal. Aristotle scarcely achieved corresponding success with modal syllogistics, and that probably explains why Prof. Bochénski gives this subject relatively little attention. The criterion throughout is that of quality, and the extent to which original minds have been able to approach perfection of presentation and methodological rigour. Included in this section are, of course, the Stoics and the Megarians, together with a host of commentators. It is good to read such a vivid extract from the tree of Porphyry, with its focus on extension and intension, and the principles of classification. This was not an epoch of discovery, but the literature is valuable and its synthetic character important. Theophrastus, pupil and successor of Aristotle, receives more than honourable mention. In addition to his logic, we salute him as a pioneer in meteorology.

Scholastic logic (Part 3) will be welcomed not only by logicians but also by medievalists. Naturally, there is a good deal here that stems from the Stoics and the Megarians. But imperfect as our knowledge of this period may be, it is one of supreme consequence since in fact such giants as Albertus Magnus and St. Thomas Aquinas brought to Aristotelianism not only a new life but also gave mankind something to live for, and thus realized, for the time being in any event, the concept of philosophy as the serving-maid of theology. This is not what is said in so many words, but the author's mastery of his material is such as to commend his scholarship to students of subjects sharing boundaries with those of formal logic.

Part 4 (classical logic) has already been mentioned. With Part 5 (mathematical logic) we are brought right up to 1931, with Gödel's famous theorem, announced in that year. The highlights here, as one would expect, are the works of Boole, Schröder, Frege, Whitehead and Russell.

Indian logic (Part 6) forms a survey of a branch of learning all too little understood in the West. Prof. Bochénski makes a point to the effect that had India produced a Plato or an Aristotle at the appropriate point in history, a long stretch of time would have been saved in the working out of principles. But this was not to be. Seen against the background of centuries, creative genius acts somewhat like a modern computer, solving almost in an instant of time some problem previously wholly intractable.

The bibliography is extensive, running to several thousand entries. It has, for reasons of economy, been photocopied from the German, a sensible plan which will hurt nobody. In addition, are a series of indexes, dealing with names, subjects and signs.

There has been nothing like this book before. It is far from easy reading, but the planning is itself so logical, and the commentaries themselves so apt, that one is left with the impression of a veritable masterpiece, set off by a frame and background at once wholly sympathetic and comely.

F. I. G. Rawlins

FUNCTION THEORY AND ITS APPLICATIONS

Lectures on the Theory of Functions of a Complex Variable

By Prof. G. Sansone and Dr. Johan Gerretsen. Vol. 1: Holomorphic Functions. Pp. xvi+488. (Groningen: P. Noordhoff, Ltd., 1960. 45 D.fl.; 12 dollars, paper bound. 48.75 D.fl.; 13 dollars, cloth bound.

MODERN mathematics is in the process of becoming more and more abstract. We are in general less interested in numbers than in spaces of objects having certain properties. However, this process of abstraction necessarily moves more slowly in disciplines with physical applications.

It is for this reason that Sansone and Gerretsen's *Lectures on the Theory of Functions of a Complex Variable*, although a little old-fashioned, is bound to make a lot of friends, particularly among those who wish to learn function theory in order to use it. The authors claim to address those interested in advanced methods of analysis. They rather quickly cover the foundations, including Cauchy's theorem, and concentrate on what one can do once the basic theorems are proved. There is, however, a fair amount of theoretical stuff such as, for example, the Vitali convergence theorem, the Borel–Caratheodory inequality and the theory of integral functions of finite order. Picard's theorem for functions of infinite order and subharmonic functions are not covered. Conformal mapping, both theoretical and practical, is left for a second volume.

Among the practical topics which are covered are the inversion of power series, theta, elliptic, gamma, Legendre and the Riemann zeta functions, Laplace integral, Dirichlet series, as well as some other special functions but not Bessel functions.

The many applications include the Euler–Maclaurin and Poisson summation formulæ, and the analytic proof of the prime number theorem, asymptotic expansions of various kinds, use of the Bernouilli numbers, summability of power series, by the methods of Borel and Mittag-Leffler, and special series of many kinds, including one for Euler's constant.

The book should be very valuable to anybody who is interested in function theory, mainly in order to use it. The English is nearly always good. The term "monotonously increasing functions" should appeal to many students.

W. K. Hayman

Woody Plants of Ghana with special reference to Their Uses

By R. F. Irvine. Pp. xcv + 868 + 34 plates. (London: Oxford University Press, 1961.) 105*s*. net.

THIS beautifully produced book, illustrated by 142 full-page line drawings and 34 plates, is primarily concerned with the useful properties of Ghana's trees and shrubs. It incorporates much of the material in Dalziel's *Useful Plants of West Tropical Africa*, and supplements it with Dr. Irvine's own extensive knowledge of this subject. There is a wealth of fascinating information on economic products and medicinal properties, both factual and fancied, for the 'doctrine of signatures' is implicit in much of African medicine. This is ably supported by a comprehensive series of indexes and glossaries, and by an extensive collection of vernacular names.

The purely botanical part of the book is less happily conceived. Each species is accompanied by a short botanical description, but this is quite inadequate to enable the species to be identified and there are no keys, while the errors in nomenclature are numerous. The citation of individual specimens seems unnecessary in a book which is not primarily a taxonomic work, and it is surely pointless to cite unnamed species known only from incomplete specimens when they have no known uses.

It is understood that this volume is to be followed by another on the herbs of Ghana. It is suggested that much of the botanical detail might profitably be omitted, leaving the author free to concentrate on the useful properties where he is clearly an expert, and enabling the publishers to offer the book at a cheaper price.

W. D. CLAYTON

Re-educative Treatment of Suppression Amblyopia

Being an abridged English version of Jean Sédan's "Post Cure de l'Amblyope Rééduqué". By T. Keith Lyle, Cynthia Douthwaite and Jill Wilkinson. Pp. xii + 135. (Edinburgh and London: E. and S. Livingstone, Ltd., 1960.) 25*s*. net.

THIS is an English version of Dr. Jean Sédan's well-known compilation of test types and pictorial exercises designed for use in the treatment of amblyopia associated with uniocular strabismus. Dr. Keith Lyle, who was responsible for the preparation of this version, states in his preface that he has found Sédan's methods of the greatest value in the work of the Orthoptic Department at Moorfields Eye Hospital, London.

Three types of exercise are represented. In the first, the patient is required to detect misprints in test passages in a variety of sizes of print. In the second, he has to solve simple problems or detect errors in exercises presented in numerical, diagrammatic or pictorial form. (Some of the pictures are delightfully French.) In the third, there is a number of special exercises intended for patients with eccentric fixation or related disturbance of spatial localization.

It is unfortunate, perhaps, that no evidence as to the efficacy of these procedures is presented. None the less, Sédan's methods would seem very well worthy of trial in the treatment of squint and related conditions in which systematic eye-exercises are prescribed. Some of his material, too, might find useful application in the study of other types of visual disorder, in particular the defects of perception which may result from injury or disease of the brain.

O. L. ZANGWILL

Inductive Probability

By John Patrick Day. (International Library of Philosophy and Scientific Method.) Pp. xvi + 336. (London: Routledge and Kegan Paul, Ltd.; New York: The Humanities Press, 1961.) 40*s*. net.

THE author of this book has performed a most valuable service. This consists essentially in treating his subject philosophically rather than in the more 'restricted' sense as an essay in logic. That little mathematical knowledge is required of the reader is neither here nor there: the approach is not esoteric. Probability as a concept is used by many professional people, but even ordinary mortals use it quite widely. Much the same can be said of evidence. The writer comments on the advantages and disadvantages of the jury system: on balance, it comes out on the right side, in spite of the fact that sheer quantity of evidential material is a strain on the layman. This is only another example of our national inclination to keep the expert in his place.

The scheme of the present volume comprises a full discussion of inductive probability, subject–predicate formulæ, functional formulæ and determinants, theory of elimination, inductive reasoning and inductive logic. In spite of a commendable determination to write clearly, the author seems occasionally to share with no less a person than John Ruskin a tendency to make meanings obscure by overemphasis on detail. Also, his use of the word 'generally' (for example, on pp. 51, 58, 72) is not altogether happy; supposedly he means 'usually'.

All in all, scientists would do well to read this book carefully, both for its meticulous scholarship and for its discipline. Their weighing up of what is true and what is likely to be true will be greatly helped, with corresponding confidence in their conclusions.

F. I. G. RAWLINS

Crystallization

By Dr. J. W. Mullin. Pp. ix + 268. (London: Butterworth and Co. (Publishers), Ltd., 1961.) 60*s*.

THE first half of this book surveys ably, with much detail, the physical chemistry, properties and phase equilibria of crystals and solutions with some reference also to melts. The final chapter summarizes in useful fashion the techniques and problems of sizing and size analyses. In between we have a chapter summarizing briefly the better-known theories of the mechanisms of nucleation and crystal growth. It would be a superhuman task to render the literature on these topics into a single, readable chapter and the author might well have condensed his material yet more without loss. As it stands, however, it is slightly incongruous to find 103 papers, presented at three symposia, dismissed in a single reference as "able summaries", while an almost irrelevant reference to gas absorption merits a whole paragraph and the beard of the late W. H. Perkin a whole sentence.

It is particularly significant that the remaining chapters, treating re-crystallization, industrial crystallization and equipment, are wholly qualitative. This characterizes the status of crystallization as a unit operation. Due to the difficulties involved and perhaps to the guarding of technical secrets, almost no quantitative investigations of the process have been published. The real utility of this book depends to a large extent, therefore, on engineering design data being made available for the process chemists and chemical engineers for whom it is intended.

S. H. BRANSOM

XUM

THE INSPIRATION OF SCIENCE*

By Sir GEORGE THOMSON, F.R.S.
Master of Corpus Christi College, Cambridge

WE are all well accustomed to science as the basis of technology, the source of the fundamental knowledge which it is our duty to apply to the service of man, but there is a quite different side to it and one not less in importance, that is science as truth, or at least one of the great aspects of truth, science as a human value—something which exists for itself and is self-justified as is music or painting, or indeed the human relations. This side should be an inspiration to all who are concerned with science in whatever form. Surely we have reason to be proud that such an insignificant creature as man should even be aware of his own insignificance? That he should be able to reckon up the countless billions of stars in the Galaxy and of galaxies in the universe and understand something of how they are made, that he should be able to measure the atoms and to determine their structure, that he should be able to understand even a little of the astonishing meaningful complexity that goes to make life? But besides its emotional appeal science is the philosophy of our age, and we who practise it should spend a little time in thinking of it from this point of view and seeing in what ways it differs from some of the older philosophies.

First of all physics relates to the outside world; it requires that a consensus of opinion be possible between different observers, or in other words that the observations made by different people can be adjusted so as to be consistent with a rational theory. One need not go into the complications of relativity, it is enough merely to consider the different judgments of relative position which two people sitting in an ordinary room will make and which are, normally at least, consistent with the furniture and the room being rigid objects and with light going in straight lines. It is in fact precisely this which is the justification for believing in an outside world: the fact that one can describe it in a way that can be accepted by all observers. Compare this outside world with the private world which each conscious person carries about with him. In this private world are dreams, emotions, pain and so on, things which he may be able to explain by symbols to his fellow men but for which, in the last resort, they must take his word and which they cannot directly share.

Physics, like other sciences, is based on concepts which have names, things such as mass, temperature, energy, rays of light, electrons and so on. It is these concepts which determine the stage to which physics has arrived because they determine what statements can in fact be made, and major advances in physics are, to a great extent, discoveries of the right concepts.

Concepts are usually suggested by experiments, but they seldom remain directly tied to them, at least not to simple experiments. Temperature is a good example. Starting from sensations of warm and cold, which (in so far as they measure anything) measure a rather complicated mixture of the temperature and conductivity of the substance in contact with the skin, temperature was early measured by the expansion of solids and liquids. These expansions are not uniform, and the scale of temperature derived from one body is near to but not identical with that derived from another. If temperature is to be a fundamental concept it must be removed from this dependence on the particular substance. It is well known how this has been done, by making it dependent on the ideal efficiency of certain heat engines; but this is not, of course, the way that anybody ever actually measures it.

Much the same kind of thing happens with space; spacial measurements, and most of geometry, depend on the assumption of rigid bodies to act as measuring rods or theodolites. There are no rigid bodies, but it is possible to make satisfactory corrections for the lack of rigidity of the nearly rigid ones. Some concepts are even less closely connected to experiments than these: for example, that of molecular chaos. It was a long time before this could be directly observed, yet the idea was most valuable to physics. The same is true to an even greater extent of the concept of atoms, and one of the lessons of the history of science is that one should beware of the purism which demands very close connexion between a concept and what is at the moment experimentally verifiable. One of the most interesting things in theoretical physics is the way in which certain concepts develop a usefulness far beyond that for which they were originally intended. Atoms are a case in point—so are quanta which, starting from a theory of black-body radiation, are now fundamental to all physics.

But not all concepts conform exactly to Nature; they are approximations, but they are not to be despised on this account. Take, for example, rays of light; they are still more important in practical applications of optics than are waves or quanta, although even in the time of Newton it was known that they were not the whole story. Another example is the use in physics of the concept of a surface as a mathematical discontinuity between two bodies, for example, solid and gaseous. There is no such thing as a surface in this sense, speaking strictly, and it is certainly not mathematically smooth. Atoms do not have sharp edges; there are vague regions round them in which they exert force, yet the concept of a surface is of immense value not merely to engineers but also to most of classical physics. Some concepts are absorbed by others, or perhaps one should say become tributary to them, for they still continue to be used, as the concept of mass has to that of energy.

There has been much discussion as to the 'reality' of certain concepts, for example of the electron. I think sometimes this fails to realize how artificial are the concepts of ordinary life, of common-sense things. Few people think it necessary to justify the idea of a chair or to ask whether it is real, yet it is in fact a most complicated idea. It is derived from a variety of different impressions, visual and factual, and is defined partly by appearance and partly use. A chair seen by the engineer is a collection of stressed material; seen by the botanist, if it is

* Substance of the first Thomson Lecture of the Society of Instrument Technology, delivered on October 19.

wooden, a collection of plant cells ; seen by the housewife, something on which people can sit. While electrons are countable so that in any given circumstances they are a whole number, in a group of chairs there may be some broken ones of which it is doubtful whether they rank as chairs or not. The chair is really a very vague idea, and if one must argue about reality (which perhaps should be considered a bad word) the electron's claims are, I would say, better than the chair's.

Concepts are used to form hypotheses, theories, and laws which are statements of varying certainty about concepts. It seems to me that the differences between these words lie more in the emotional attitude of the user than in anything else. A law is some statement about concepts in which you really believe; a hypothesis is one about which you choose to remain uncertain. I will just use the middle word 'theories', the emotional content of which is somewhere between the two. All physics is based on experiment ultimately, and on the idea that experiments can in some way be repeated. By experiment I do not necessarily mean something in a laboratory, but merely something that can be observed ; for this purpose the explosion of a supernova is an experiment. The first point that I want to make is that one cannot repeat an experiment unless one has some sort of theory, because repeating an experiment implies that the conditions are the same on the two occasions. Now all the conditions are never the same: life moves on, the planets are in different positions, the apparatus is either made of the same bits of material (in which case it has been worn) or is made of different material, in which case how do you know it is effectively the same? The answer is, of course, to say that the relevant conditions must be the same if you wish to repeat an experiment. But who is to say which conditions are relevant and which are not? This can only be done by a theory. The theory need not be a detailed one, it may merely say 'I believe that all that matters for the experiment I am now going to perform is the state of the objects on this table' and this may well be true if you make some allowances for the gravitational direction of Earth. But your experiment may go badly wrong if someone is making electrical disturbances in the next room. The theory must be sufficiently wide to give you an idea as to what matters.

Dr. K. Popper has made the very good point that theories in science exist to be disproved ; they can never be proved. Since they are general statements that something will always occur, one can never be certain that a case of exception will not occur ; if it does occur the theory is disproved and is either abandoned or, if it has shown previous merits, is somehow altered to fit the new evidence. The great strength of science as compared with philosophy is that theories can be disproved or at least shown as inadequate and that this can be done in a manner which compels the assent of all, or almost all, reasonable people ; in short, that a consensus of opinion is possible and one which appears to be lasting. This consensus, philosophy never seems to have achieved, or at least only for short periods.

Personally, I think that the existence of technology is an enormous support to theoretical physics in this way. It is impossible not to believe in the validity of theories which enable complicated machines like television sets to be made and operated by people who are wholly ignorant of the scientific principles which we scientists say they depend on. It seems to me psychologically impossible not to believe that such principles have, in fact, a validity external to our minds. It is not necessary for this purpose that the principles are absolutely true or extend over all conditions ; it is possible that Maxwell's equations do not apply in the interior of an atomic nucleus, but this does not detract from their validity for television.

Of course, the prestige of a theory goes up enormously if it can predict the unexpected, and rightly so. The mere fact that a theory covers known experiments counts for very little; after all, it is possible to draw curves through any finite number of points in an infinite number of ways, no one of which is in principle better than another. Experiments can always be represented by empirical formulæ which need have no further validity whatever. A theory must be æsthetically satisfying as well as fit the facts. This is equivalent to saying that Nature in some way respects our æsthetic judgment. In a sense I think it does, but this is very largely because our judgments of what is an æsthetically acceptable theory are based on our experiments on theories of the past which have been successful. In other words, we are assuming that Nature is somehow of a piece, and that we can hope to recognize theories which conform to the pattern just as one might hope to recognize somebody's handwriting in a few words though one has never seen those actual words written by him before. I am not sure whether this implies a personification of Nature or whether it is a consequence of the way our brains work.

A good analogy of the relation between physical theories and external realities is given by an ordinary map of Earth's surface. Like the map, the theory is either something on paper or in the mind of the physicist, just as the map may be in the mind of the traveller. The concepts of physics correspond to the signs on the maps denoting rivers, roads and the like. The making of such a map consists in translating Nature on to a piece of paper, and the use of the map involves reversing this process, reading the map, deciding, that is to say, that one particular point on the map is the place where you are standing, that the hill on your right is represented by a certain group of concentric contour lines on the map and so on. All theories are approximate in practical application, ignoring small effects ; just so, all maps leave out details. They may show hills and slopes but they will not show molehills. This, however, does not prevent them from being useful. Again, every map of Earth on a flat piece of paper is necessarily distorted since you cannot bend a plane to fit a sphere. Several kinds of maps are possible corresponding to the different methods of projection; some are more suitable for one purpose, some for another, but there is none which is universally the best. Yet a map can be right or wrong ; a map which shows Piccadilly Circus north of Oxford Street or Birmingham in the middle of Spain is definitely erroneous.

This applies to scientific theories. They can be so badly wrong as to be useless. They can never hope to be completely right, with the rarest and doubtful exceptions. Two quite different theories may be equally valid : compare for example, the thermodynamics and the kinetic theory approach to the properties of gases ; they start from wholly different ideas yet arrive at practically the same conclusions, but for some purposes one is better than the other.

The great authority of Lord Kelvin is often quoted to extol the importance of the quantitative, which

implies measurement, in physics. He was, of course, right in urging its importance ; but it should, I think, always be remembered that in the last resort we are trying to do something qualitative. The pure scientist is aiming at producing a theory which will be convincing and satisfactory, which will make people feel that they understand, and which will predict something new and exciting. The object of the applied physicist or engineer is also in essence qualitative. For example, if he is designing aeroplanes the question of top speed is very important and it can be measured. But speed, predicted or measured, is important because of its qualitative implications. You desire an aeroplane which will get you to your destination before your friends are tired of waiting for you, or if it is for war, one which will return safely from its sortie having shot down the enemy. The quantitative predictions, important as they are, are really the means to the end, not the end itself.

I have spoken of a good theory being æsthetically satisfying because as we have seen a mere empirical formula is hopeless. Comparatively little has been written on the æsthetic side of science apart from mathematics. Some of the same criteria apply here as do there: for example, the theory must be general, simple and striking. It should not involve arbitrary constants or functions, unless indeed it can be shown that the constants which are introduced are related to something quite outside the theory itself: for example, Maxwell's equations and the velocity of light. But I am inclined to think that the most important feature in making one accept a new theory is what I have already said, its general resemblance to the successful theories of the past.

The quantum theory has made, as we are all aware, an alteration of immense importance in our ideas of physics. It has replaced determinism by probability. The uncertainty principle of Heisenberg is, I think, the first occasion on which science has laid down limits to knowledge as opposed to the familiar limits to what man can perform given by such principles as the laws of thermodynamics. It means an alteration in the sense in which Nature is rational. One cannot predict certainties; one can only predict probabilities and it becomes a matter of some difficulty to know what is meant by a probability when you deal with a unique event, an event which cannot be repeated—for example because the apparatus is changed or broken and is too complicated to re-make. I am thinking in particular of experiments on brains, animal or human. In more ordinary laboratory experiments one can use an apparatus or series of apparatus, each of which conforms to the same theory. This theory asserts that results a, b, and c are probable in calculable proportions, and by prolonging the experiment it is possible to verify the theory, if true, to any degree of accuracy required. Each experiment is really a quite separate one and the only feature in common is that you believe them to be representable by the same theory.

Recently Born has pointed out that quantum indeterminacy has wider implications than one might suppose. Consider, for example, the Brownian movement: In what possible circumstances can the motion of the tiny drops of butterfat seen in a microscope moving about in all directions in the liquid be regarded as determinate? Their motion in the view of nineteenth-century physics is controlled by the collisions they make with the molecules of the liquid; but who is to calculate the initial positions of the molecules of the liquid and their collisions with one another ? To make the matter a little more definite let us imagine a more precise experiment. Suppose that we have a Geiger counter divided into two halves by a grid and filled with gas at suitable pressure. Let us introduce a single radioactive atom into this counter, at a known place. The atom is supposed to stick to a wall of the counter as soon as it touches, as it eventually will. Now try to calculate in which half it will end up. It will explode sooner or later and that half will be discharged giving an observable effect. To attempt to predict this, one would have to trace the zig-zag path of the atom diffusing through the molecules of the gas. If you try to consider the accuracy with which you would have to measure initially the positions and velocities of the millions of molecules concerned you will, I think, agree that to regard such an experiment as determinate is a misuse of language, even on the simplest assumptions such as assuming the molecules to act like billiard balls. The motion in each collision is sensitive to the position of the moving balls at the moment ; a very small error in determining this will result in a big change in the direction and velocity of the balls after the collision, and so effect to a still greater extent all subsequent collisions. The errors in fact are magnified enormously, and long before the process comes to an end with the active atom reaching the wall, the initial accuracy required for prediction becomes fantastic. In the nineteenth century the physicist could say "yes, but in principle the motion is determinate". Since Heisenberg we can no longer do this ; the motion is indeterminate. But I want to point out that this conclusion depends very little on the precise magnitude of h provided it is not absolutely and mathematically zero. I think that there are probably far more examples in practice of these truly indeterminate events than one is apt to suppose. One may be too impressed by the smallness of h. Take, for example, aerodynamics, where one is concerned with turbulent motion set up as an instability, as when, owing to changed speed, the laminar boundary layer of an aerofoil changes to a turbulent one. This may depend, of course, on roughnesses on the surface and therefore be determined by them, but it is quite likely if the surface is smooth that the instability is triggered off by a fluctuation, in the kinetic theory sense of the term, and it is within limits indeterminate when and where this happens. Most cases of instability are like this ; they may become determinate because of some deviation from the ideal in the geometrical shapes, but if these are accurate enough there will come a time in which wholly unpredictable micro-effects will set the instability off. It seems quite likely that details of meteorology may be unpredictable in this fashion, and even quite major features may depend on unpredictable events in the Sun. All physics can do is to predict probabilities, not merely for atomic events but also for many other classes even where large effects are concerned. There are a great many trigger actions in Nature, especially where biology comes in.

It is perhaps scarcely necessary to urge that the discovery of new effects is fully as important a test of the merits of a theory as the explanation of old ones; but some writers on the philosophy of science seem to regard the main duty of a theory as the proper ordering and correlating of known facts. This seems to me an unimaginative view. In the past, theories have gone much beyond the known facts and pro-

foundly helped physics by doing so. Maxwell's equations connected the velocity of light with the ratio of electrostatic and electromagnetic units, but they also suggested waves of very different lengths, some of which Hertz discovered, and the theory also inspired von Laue to try X-ray diffraction. The idea that many crystals are formed by the close-packing of nearly spherical atoms was put forward by Pope and Barlow; their theory was wrong in detail, but suggested to Bragg ideas now fundamental in the study of crystals.

Imagination indeed is one of the qualities which mark the great scientist. Even a hint of it may lift a man out of the rut. There is only one quality which is more important and that is enthusiasm, the feeling that science is the one thing that really matters and that to be allowed to spend one's life in its pursuit is the greatest possible privilege.

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION, AUSTRALIA

DIVISION OF FOOD PRESERVATION: NEW LABORATORIES

THE Division of Food Preservation of the Commonwealth Scientific and Industrial Research Organization, Australia, has moved into new laboratories at North Ryde, New South Wales. This Division began its work in 1932 in laboratories situated in the Brisbane Abattoir, Queensland, where a branch laboratory is still maintained. In 1938 the Division transferred its headquarters to laboratories in the Abattoir at Homebush, New South Wales, but in twenty years outgrew the accommodation there.

The new laboratories are located on an elevated site, about eight miles from Sydney, in an area that is being preserved as a green belt to the city. Eleven buildings with a total floor area of 71,000 sq. ft. are set in landscaped grounds about 10 acres in extent. The disposition of the buildings on the site was determined largely by a desire to provide for future extensions to individual buildings without interference with other buildings and services.

In the centre of the site (Fig. 1) are the service facilities—workshops, stores, boiler house, engine room, and canteen, and also a Taste-Testing Laboratory, which has been carefully planned to provide a standard environment for sensory testing. Flanking

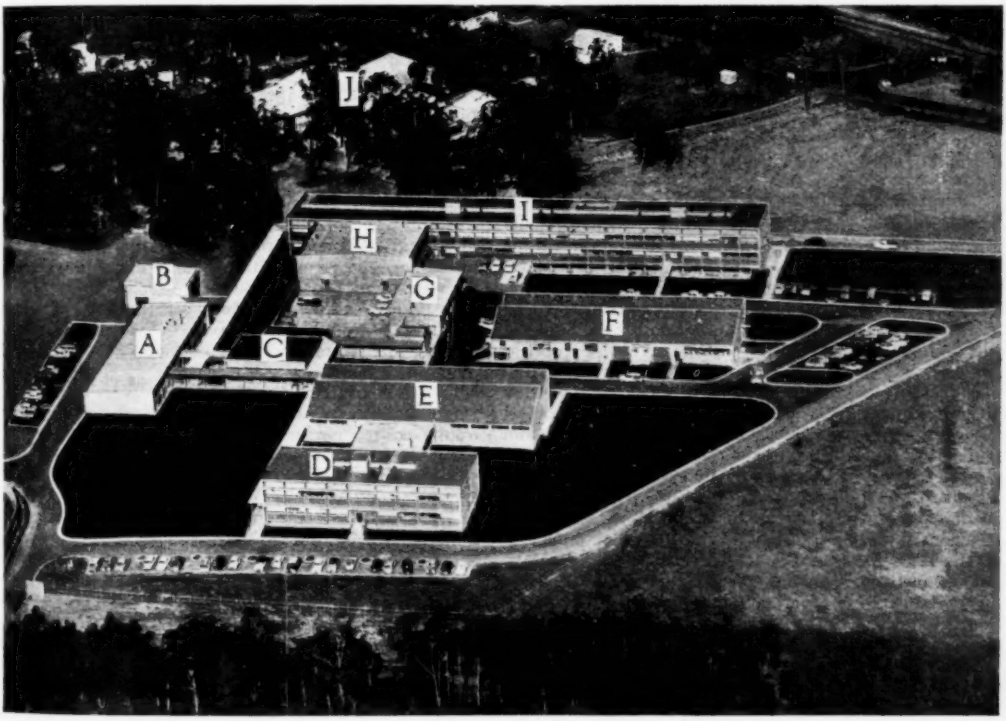

Fig. 1. Aerial photograph of the new laboratories of the C.S.I.R.O. Division of Food Preservation. *A*, administration; *B*, Hicks meeting room; *C*, canteen and Taste-Testing Laboratory; *D*, food technology; *E*, food processing; *F*, workshops; *G*, boiler house and engine room; *H*, cold room block; *I*, food science; *J*, Bread Research Institute

Fig. 2. Mural by Miss Eileen Mayo which decorates the foyer of the administrative building in the new laboratories

these buildings to the east are a single-storey building housing administration and the library, and the E. W. Hicks Meeting Room, named in honour of a pioneer member of the Division. On the south side of the site is the Food Science Building, which contains forty laboratories and accommodates the Physics, Chemistry, and Microbiology Sections, and also the Fresh Fruit and Vegetable Storage Section, and an Animal Products Section dealing with meat, fish, poultry and eggs. The other technological Sections of the Division—Canning, Packaging, Dried Foods and Frozen Foods—occupy the twenty-two laboratories in the Food Technology Building, and also use the adjoining Food Processing Building, where there is an open processing area 130 ft. × 50 ft., containing pilot-scale equipment for the production of experimental packs of processed foods. In a separate building is a sulphuring room for work on the treatment of dried fruits with sulphur dioxide and a chemical processing laboratory for operations involving volatile solvents.

The laboratory buildings have a central corridor with load-bearing walls in brick, and reinforced concrete supporting piers at 12-ft. centres on the external walls. Aluminium has been used for roofs and window frames, and also for the coloured enamelled spandrels, which are removable for access to the services running in perimeter ducts along the north and south walls. Penetration of summer sunshine on the long north and south sides of these buildings is controlled by continuous concrete hoods and vertical pre-cast concrete fins. The hoods also act as working platforms when access is required to the perimeter ducts.

The services available in the laboratory blocks are hot and cold water, distilled water, gas and alternating-current power, with compressed air and steam in some laboratories. Fume cupboards are provided with individual exhaust fans, and in some sections separate fume rooms containing two fume cupboards have been installed. Water and gas are run in copper pipes, distilled water in stainless steel, and sink wastes in polythene and glass piping. The library, the taste-testing booths, and several specialized laboratories are air-conditioned, while the remaining buildings are heated by hot-water radiators.

The buildings are linked by an internal road system and by covered footways which also provide overhead carriage for some services.

Two general types of layout have been used within the laboratories, according to whether the function is predominantly chemical or biological, but officers were given considerable freedom in the detailed planning. A variety of service rooms has been provided, ranging from balance and instrument rooms to a suite of preparation and sterilizing rooms for microbiological work. Many of the internal partitions are removable to permit changes in layout, and bench units and service lines can be readily dismounted and re-used. Laboratory bench tops are solid timber finished with a clear epoxy resin, while fume cupboards are finished in a white pigmented epoxy resin. Natural wood finishes have been used extensively on laboratory furniture, and in conjunction with the light-coloured walls the general effect is very pleasing.

In a wing of the Food Science Building are 31 controlled-temperature rooms with a range of constant temperatures from −30° C. to 50° C. Four of these rooms are gas-tight for the storage of food in controlled atmospheres.

Immediately adjoining the Division of Food Preservation at North Ryde is the Bread Research Institute of Australia, a research association financed by the Bread Baking industry and the C.S.I.R.O. jointly, and this Institute houses a C.S.I.R.O. Wheat Research Unit. There is thus a useful centralization of food research establishments at North Ryde.

The Division of Food Preservation has a total staff of 150, of whom 78 are graduate research officers and experimental officers, and 49 are technical assistants. The field of research in which these workers are engaged is the handling, preservation, processing, storage and transport of meats, fish, eggs, fruits and vegetables. More than half the resources of the Division are devoted to fundamental research seeking a deeper understanding of the properties of foods and the changes which they undergo during processing and preservation. In addition, therefore, to technological projects on the preservation of foods by canning, dehydration, and refrigeration, the research programme includes many projects in the basic sciences.

Present chemical research is concerned with applications of gas chromatography to volatile constituents of foods, with anthocyanin and other flavonoid pigments in foods, and with reaction mechanisms in the non-enzymatic browning of foods and its control by sulphur dioxide. Workers in muscle biochemistry are elucidating the effects of rigor mortis and freezing on the protein and other constituents of muscle. Theoretical work on physics, on heat- and mass-transfer across laminar boundary layers, are related to many problems encountered by food technologists. Fundamental microbiological studies on the effects of water activity on microbial growth and on the germination and growth of bacterial spores are designed to give precise information on factors governing the control of micro-organisms which cause food spoilage and food poisoning. Studies in plant physiology on the metabolism of carbohydrates and organic acids and on the properties of plant cell membranes are related to outstanding problems in the preservation of fruits and vegetables.

The new laboratories were officially opened on September 18 by Dr. D. A. Cameron, Federal Minister for Health and for the C.S.I.R.O., in the presence of a large gathering of representatives of Government and university departments and the Australian food industry. On the four days following, a Food Science Conference was held and was attended by 250 Australian food scientists and technologists and a few overseas visitors. The programme included symposia on "Chemical Aspects of Food Processing" led by Prof. C. O. Chichester of the University of California, Davis, on "Meat Quality" led by Dr. E. C. Bate-Smith, Low Temperature Research Station, Cambridge, and on "Food Preservation and the Organization of Plant and Animal Tissues" led by Dr. J. B. Biale, University of California, Los Angeles, and also panel discussions on public health aspects of handling and processing foods and on trends in food research.

UNCONVENTIONAL METHODS OF ENERGY CONVERSION

A JOINT session of Sections A (Mathematics and Physics) and G (Engineering) of the British Association was held in Norwich to discuss the present situation with conventional methods of converting heat into electricity, the need for new methods, the principles of the new methods on which research is in progress, and the prospects for the future.

In the opening paper, Prof. T. G. Cowling (University of Leeds) pointed out first that with the established methods of using solar energy indirectly, by fossil fuel or by water-power, the proportion of the incident energy which is utilized for energy conversion is extremely small. This proportion is nevertheless, up to the present, readily available. The nuclear fission process is now well established, and can be controlled and made available satisfactorily. The direct employment of solar energy is, however, difficult because, although 1·5 kW./m.^{2} arrives from the Sun outside the Earth's atmosphere, the problems of concentration mean that it is not readily available. Even greater difficulties with availability of the energy apply, of course, to thunderstorms, and so far to nuclear fusion processes. Thus, there are no new sources of energy likely to compete for some time with the existing sources. For new methods of utilizing existing sources of energy, those of interest appear to be thermoelectricity, the thermionic diode, magnetohydrodynamics and the fuel cell. The last of these was omitted from this session, and dealt with in a meeting of Section G on the following day.

Mr. F. H. S. Brown (Central Electricity Generating Board), in the second paper, considered the incentives for establishing unconventional methods of energy conversion. The conversion of mechanical energy to electrical is now very efficient indeed, and the problem remaining with conventional methods is the conversion of heat to mechanical energy. This is, of course, limited ultimately by the efficiency of the Carnot cycle, though the best efficiency obtained is only about 70 per cent of this. To raise the efficiency of the Carnot cycle requires an increase in the temperature at which heat is fed into the cycle. With present materials, no engine with highly stressed moving parts can be operated continuously at a temperature much above 600° C. Thus, although a fuel may be burned at 1,700° C., no such temperature can be fed into an engine continuously. In the internal combustion engine, the explosion produces a high temperature which falls rapidly during adiabatic expansion, thus lowering the mean temperature of the engine parts. However, there is a limit in practice to the size of the internal combustion engine, it is expensive, and moreover, since it is of the reciprocating type, it suffers from friction, wear and noise to an unacceptable degree.

The steam cycle is thus more satisfactory, although it has a limiting temperature near 600° C. The efficiency obtainable is about 40 per cent, and in this case, unlike the internal combustion engine, scaling up improves the performance obtainable. The gas turbine involves again continuous exposure to the maximum temperature, the limit of which at present is near 600° C.; but a better performance can be obtained compared with the steam cycle because of the lower pressure and the larger heat rejection at the minimum temperature of the cycle.

It is clear that it would be very desirable to increase overall efficiency above 40 per cent, and the methods of achieving this could in principle be: (1) finding an intermittent process cheaper and more reliable than the internal combustion engine; (2)

evolving better materials for use at high temperatures, for example, up to 1,700° C.; (3) evolving a continuous method which does not stress working parts at high temperatures.

In the third paper, Dr. R. G. Siddall (University of Sheffield) described the work of Prof. M. W. Thring and himself on producing electricity directly from fast-moving plasmas. Here the ionized gas of the plasma replaces the conductor in a dynamo, and moves through a magnetic field, converting its kinetic energy directly into electricity. To obtain useful power, the plasma must have an electrical conductivity near 1 ohm^{-1} cm.$^{-1}$, and it must move at several thousand miles per hour through a magnetic field of 10,000–20,000 œrsteds. Moreover, the path-length through this field to extract the energy must be many feet. Ordinary gases would have too low a conductivity at any feasible temperature, but by 'seeding' with a small proportion (say 1 per cent) of the vapour of an alkali metal, temperatures near 2,500° C. would be sufficient. The simplest scheme would be to preheat air, compress it and mix with a fuel, burn it at about 2,500° C., and expand through a nozzle to obtain the velocity. Unfortunately, this would cool the gas almost to 1,000° C.; to maintain it near 2,500° C. after expansion would need a combustion temperature near 6,000° C. Thus, more sophisticated schemes are necessary.

Three ideas due to Prof. Thring are under investigation. First, continuous combustion at 2,500° C. followed by expansion through a nozzle is used to produce the high velocity. Secondly, short bursts of preheated oxygen and fuel seeded with potassium are injected periodically into the combustion chamber immediately before the expansion nozzle. This burning gas is swept along in 'slabs' by the main gas stream. These slabs are expected to retain their initial temperature during their passage through the magnetic field. The second scheme involves obtaining the high velocity without pre-compressing and expanding. The combustion occurs at constant volume, in a chamber with inlet and exit valves which achieve pulsating combustion, so that again the hot gas passes in slabs through the magnetic field.

The third idea utilizes shock waves produced successively at the opposite ends of a tube, so driving a plasma to and fro through the magnetic field. This will generate power at each passage, and will produce alternating current. It is thought that these methods, if successful, will produce efficiencies of 50–60 per cent.

Mr. P. Dunn (Atomic Energy Research Establishment) described, first, the basic principles of the thermionic diode as an energy converter, using the analogue of the steam boiler to illustrate the electron evaporation from the cathode and collection at the anode. He showed that for a given cathode operating temperature, the cathode work function should be below a maximum allowed value which is a function of the operating temperature, but that there is no optimum value. He indicated the importance of low anode work function, and of minimizing the effects of space charge either by using very close-spaced vacuum diodes or by positive ion neutralization of the space charge. The former method is impracticable for generation of large amounts of power, though it is valuable for space vehicles. With cathodes operated at 1,400° K., present diodes give an output of 0·7 V., 1 amp./cm.^{2}, with an efficiency near 15 per cent. Higher outputs and efficiencies will be obtained with higher cathode temperatures. For use in a reactor, supposing that the fuel elements could be run at temperatures up to 2,000° C., but not higher, a cathode with a work function less than 3 eV. would be required. This cannot be a metal, and none of the known oxides used as cathode materials is satisfactory at 2,000° C. Uranium carbide has, however, a work function near 3 eV., and may be useful both as cathode material and, suitably enriched, as the fuel element. Using cæsium vapour to give the positive ions, this should maintain a cæsium coverage on the anode which would give a reasonably low anode work function. It is not certain yet whether the cæsium would be adequately ionized in such a diode, and it is known that there are considerable difficulties in using cæsium due to attack of other metals, glass and ceramics. Thus other forms of ion injection are under investigation.

The thermionic diode is conveniently used with fission nuclear power, as it is easy to obtain the required heat flux to the cathodes. To produce a plasma jet would not be easy with a reactor. Conversely, with fossil fuel, it is not difficult to produce such a jet, but the heat flux to a cathode is difficult to obtain. The thermionic diode is suitable for 'topping', in that the heat injected at the cathode at 2,000° C. is conveniently rejected at the anode at temperatures of 400–600° C., so that it could then be used in a conventional steam cycle. The thermionic diode therefore promises a useful step forward under the third heading above. Incidentally, the high-velocity plasma cannot be used after the hottest part of the gas has cooled below about 2,000° C., so that this method could also be used for topping.

In the final paper, Prof. D. A. Wright (University of Durham) discussed thermoelectricity as a means of generating power directly from heat. He first outlined the basic principles, showed why the performance of metallic thermojunctions was poor, and contrasted the possibilities with semiconductors. With the latter there are many complications, but the main requirements with the 'broad-band' type are that they should have a sufficiently high energy gap, depending on the required temperature of operation; that it should be possible to prepare them n-type or p-type, with a Seebeck coefficient α near $\pm$ 200 μV./deg. C.; and that they should then have a high electrical conductivity σ and a low thermal conductivity K. The usefulness of a material is, in fact, determined by the quantity zT, where $z = \alpha^2\sigma/K$ and T is the absolute temperature. It would be necessary to prepare the material reliably and repeatably, reasonably cheaply, with a high degree of uniformity of properties over every sample, from sample to sample, and from batch to batch. It must be possible to make low-resistance reliable contacts, and the material must not melt or decompose in operation; the deliberately added impurities must not diffuse out, and impurities from the contacts or from the environment must not diffuse in.

One of the best materials so far is bismuth-antimony–telluride, which has a value of zT slightly greater than unity, but cannot be operated above 150° C., because of its low-energy gap. With the hot junction at 150° C., cold junction at 0° C., a thermojunction between p- and n-type material would give a junction efficiency near 7 per cent. Lead telluride can be operated at 500° C., but with zT less than unity, so that the junction efficiency between 500° C. and 0° C. is about 12 per cent. Unfortunately, the figures realizable in practice are lower than these because of resistance at contacts, temperature drop

at contacts, and, with fossil fuels, problems of heat transfer to the hot junctions following combustion.

Progress in finding materials which can be operated at higher temperatures is slow because there are incompatibilities in the requirements. To obtain higher energy gaps and so permit higher operating temperatures one needs compounds of lower atomic weight which, compared with bismuth and lead tellurides, tend to have higher thermal conductivities and lower values of the electrical conductivity for a given Seebeck coefficient. Thus the trend is for materials which can be operated at higher temperatures T to have lower values of z, and in fact zT, than materials which can be operated only at low temperatures. Indium arsenide containing gallium or phosphorus can be operated to about 700° C., but zT is only about 0·5. More promising are some compounds such as $AgSbTe_2$ and its analogues formed by cross-substitution from the same columns of the Periodic Table. These have lower thermal conductivity than their mean atomic weight would predict. Values of zT as high as 1·5 up to 700° or 800° C. seem likely to be attained with these compounds. Materials which could be operated to 1,100° or 1,200° C. have so far lower values; for example, cerium sulphide, zT near 0·5, and nickel oxide, zT still lower. Thus the highest junction efficiency which is theoretically obtainable at present between 1,200° C. and 0° C. is about 17 per cent, while the best actually obtained so far for this range is only about 6 per cent. This is in a 'cascaded' system in which three or four stages are used, the heat from the first stage passing to the second and so on, and different materials being used in the different stages, to match the temperature involved.

To obtain useful performance for 'topping' in power generation, values of zT near unity would be necessary at temperatures up to 1,600° C., while for direct replacement of present generators operating up to 600° C., values of zT would have to exceed 4. There seems little prospect at present of obtaining values as high as this in either temperature-range. Meanwhile, research is continuing, and this type of generator is useful in spite of low efficiency for the production of low power in portable equipment and in remote situations where such a generator has to run unattended for long periods.

D. A. Wright

THE INTERNATIONAL COUNCIL OF SCIENTIFIC UNIONS

THE ninth assembly of the International Council of Scientific Unions (ICSU) met in London during September 25–28 under the auspices of the Royal Society. About 130 delegates attended, each representing a particular scientific discipline or organization. They came from all continents, and all, to quote Prof. A. V. Hill, were working scientists of the world. If the Council were a political union, it might be better known. In his welcoming address Sir Howard Florey described it as a living example of voluntary international co-operation by scientific men themselves in the best use of science to serve a world community, and in educating the public about the real purpose of scientific knowledge and research. Many changes in the modern world, although often described as social or economic, may stem more directly from the growth and applications of science itself. To say, however, that science has now become so big that it is a matter for Governments alone is a dangerous half-truth, for freedom of thought and objective analysis are essentials which must be preserved, unencumbered by an excessive bureaucracy or by considerations of political expediency. The principles and aims of science are not national but universal. International co-operation is no longer just a matter of nomenclature, symbols or standards; we have reached a stage when measurements on a particular phenomenon may be useless unless made simultaneously at widely separated places. We must also consider, for example, the effects of space research or of nuclear explosions not only on the future progress of scientific research on the cosmos itself but even more specifically on the world as a place for humanity or any other biotic community. No observer at this assembly could fail to sense in this distinguished gathering an underlying realization of responsibility by scientists themselves of all lands, to apply their knowledge and such influence as they may possess for the benefit of all mankind.

The International Council of Scientific Unions was established in 1931. Its precursor, the International Research Council, had been set up in 1919 but proved unsatisfactory because it was restrictive, in no sense fully international, and because it allowed feelings engendered by the First World War to cloud its proper function. These mistakes were avoided after the Second World War, and the recent assembly reaffirmed its previously declared non-political philosophy by admitting to membership under the terms of its rules Hungary (Academy of Sciences), North Korea (Academy of Science, Phyongyang), South Korea (Academy of Science, Seoul), Ghana (Academy of Learning), Ceylon (Association for the Advancement of Science), and East Germany (Berlin Academy of Sciences).

The Council has several objects. It aims to co-ordinate the activities of the international scientific unions, and to be a co-ordinating centre also for the national organizations which are in membership. It seeks to encourage scientific activity in fields which do not fall directly within the scope of any existing organization, to enter through the national organizations into relations with Governments and to maintain relations with the United Nations and its Specialized Agencies. The Council consists of national members and scientific members. There are now fifty-one national members, each representing the authoritative scientific organization in the country concerned—an academy, national research council or similar institution or, in a very few cases where no appropriate body exists, the Government itself. Fourteen international unions are now members. Of these, the general unions include astronomy (IAU), biology (IUBS), pure and applied chemistry (IUPAC), geodesy and geophysics (IUGG), geography (IGU), pure and applied physics (IUPAP), mathematics (IMU), and geology (IUGS), which was admitted at the recent Assembly. The specialized unions include crystallography (IUCr), history and philosophy of

science (IUHPS), theoretical and applied mechanics (IUTAM), scientific radio (URSI), physiology (IUPS), and biochemistry (IUB).

ICSU has neither the wish nor authority to interfere with the autonomy of these unions, nor to disturb the work which they are specifically designed to undertake. The range of their work is enormous. It includes the arranging of international symposia on matters of universal importance, the preparation of reports by specially appointed commissions, and the instigation and organization of research which requires international collaboration. For example, IAU has dealt with atomic time standards, and has helped astronomy and space research. Through its activity, also, many astronomers have been enabled to obtain the use of instruments in countries other than their own. IUGG has concerned itself with problems of Earth and its atmosphere, such as earthquakes and volcanoes, the atmospheric absorption of solar radiation, and the levels of oceans and the penetration of radiation into them. The data which it has collected are published and available to anyone. In the future it will participate in projects of the International Committee on Geophysics (CIG) discussed later. IUPAC has recently published reports on nomenclature and terminology, on internationally agreed analytical standards, on problems of foods and fats, and in collaboration with IUPAP has laid down a revised scale of atomic masses based on carbon-12. IUBS has dealt with quantitative methods in pharmacology and toxicology and with problems of cellular biology, embryology and genetics, and IUB has made a complete survey of known enzymes. URSI has been considering the results of the International Geophysical Year (IGY) in relation to the ionosphere, problems of radio communications in space, and has given advice about the allocation of frequency channels. IUTAM has studied the problems of atmospheric pollution, and IGU has set up groups to deal with new world maps, erosion and coastal geomorphology, and the problems of humid tropics or arid zones.

The function of ICSU is to co-ordinate and integrate the work of the different disciplines, and to catalyse research in fields not covered by the separate organizations. For the latter purpose, special committees have been set up, supported in the early stages by funds from ICSU, although each must later find support through the participating national organizations. A strikingly successful example was the International Geophysical Year, in which 67 countries took part, some 2,000 observing stations being established, 57 of them in the Antarctic. The results accumulated during this project are still being analysed, but publication will soon be complete. In order to exploit this success, the International Committee of Geophysics (CIG) was set up, with more than 60 national committees, the appropriate unions and the World Meteorological Organization taking part, to maintain the operation of the World Data Centres, to ensure further inter-disciplinary co-operation and to arrange universal publication of the relevant scientific data. Its future plans include a World Magnetic Survey, the study (1962–65) of Earth's Upper Mantle—a 1,000-km. crust, and in 1964–65 the International Year of the Quiet Sun (IQSY). IQSY will obtain data on geomagnetism, aurora, airglow, the ionosphere, solar physics, cosmic rays and space research during a period of sun-spot minimum, complementary to those obtained in the IGY period of sun-spot maximum.

Another special committee is SCOR, dealing with oceanographic research of all kinds. There is the effect of circulation on the welfare and migration of the plankton, the spread of radioactive wastes, the study of surface waves in relation to the design of ships or to erosion, and new methods of studying the sea floor. More specifically SCOR has planned for 1963–64 an Indian Ocean expedition, in which at least thirteen countries and more than forty ships are expected to co-operate, and for which a good deal of reconnaissance has already been completed. The expedition will make measurements on marine geology, geophysics and bathymetry, physical, chemical, and biological oceanography, and meteorology from the air-sea interface to the upper atmosphere. The results may lead to a better understanding of the natural factors which affect the large population living in countries bordering on the Indian Ocean.

The Special Committee on Antarctic Research (SCAR), has continued work along the lines of the IGY, involving a long-term programme of measurements at many stations on a variety of geophysical matters. Twelve nations are at present taking part, and the agreement by treaty to freeze all territorial claims for some thirty years shows a high degree of co-operation. Advice sought from SCAR by several Governments has been freely given.

Another descendant of the IGY is the Special Committee on Space Research (COSPAR) which aims to use rockets or rocket-propelled vehicles for carrying out scientific research in the widest sense. In the public eye, space science is loaded with military and commercial interests and also provides some satisfaction to sensation-hunters. It is most important, however, to preserve in proper perspective unbiased space research on a world-wide basis and to ensure that the top scientists concerned are maintained in personal contact. COSPAR, first set up by ICSU in 1958, had many problems in its first year, but under an agreed charter (1959) can now hope to perform a valuable service. It is not concerned with technical problems of rockets or with manned space flights. Ten of the international unions and 16 national members are affiliated to it, and relations are maintained with the United Nations, the World Meteorological Organization, and the International Telecommunications Union, all Governmental bodies. COSPAR is only just beginning to achieve its object, but two examples may serve to illustrate its potential value. First, if the science of radio astronomy and of space research itself is to be continued at all, some frequency bands must be kept free from interference. The 21-cm. band has at present been exclusively preserved, but as Earth-to-Earth communication by satellites for commercial, military or other purposes is developed, larger parts of the spectrum will be claimed. COSPAR, with IAU and URSI, must explain and emphasize the need. Secondly, space experiments are now possible, or even in progress, which may for ever prevent some aspects of proper scientific research, quite apart from their damaging effects on mankind. The creation of a belt of needles, in the project 'West Ford' was considered as possibly hampering or even stopping future work on both optical and radio astronomy. For these sound reasons, the ICSU Assembly agreed unanimously to invite COSPAR "to examine any proposed experiments or other space activities that may have potentially undesirable effects on scientific activities and observations, to arrange for careful objective quantitative studies, and to make available to unions, academies, and

Governments the facts and analyses needed by them for making wise and proper decisions concerning the proposed experiments". It further appealed to all Governments planning to launch space experiments which could possibly affect other scientific research adversely to make available to ICSU the information and data about the proposed experiments in sufficient time to ensure their proper consideration.

ICSU has other commitments. The Federation of Astronomical and Geophysical Services (FAGS) provides an international time bureau, a gravimetric bureau, and Earth tide service and a central ursigram service for the transmission of coded ionospheric, solar and geomagnetic data. The Joint Committee on Radioactivity (JCAR) provides standardization of radioactive isotopes, and is linked with the International Atomic Energy Agency. The ICSU Abstracting Board has done much to co-ordinate abstracting in both physics and chemistry. Funds are also to be sought so that a revised set of bathymetric charts of the oceans can be published. An international committee has recently been set up to examine the teaching of science at university level.

The London assembly took an important decision to initiate a world-wide programme for studying the biological basis of human welfare. The physical and biological condition of the surface of Earth is undergoing a more rapid and extensive change than might ever have been expected. Much of this change is due in one way or another to the development and application of physical and medical science. Desirable as much of this may be, some of the influences may lead to serious changes in the frequency of certain genes. Higher levels of radioactivity, too, although at an apparently insignificant dose-level, may in the long term be harmful to the progeny of man or animals or plants. We need not only to investigate these problems, but also to educate the public on how to preserve satisfactory world conditions. ICSU has therefore resolved to study, through the Unions of Biology, Biochemistry and Physiology, the effect on living communities of changes in natural environment, and the augmentation through basic research of natural resources and the reduction of losses and waste. It is hoped to examine the somatic and genetic variation induced by environmental factors on man and other organisms, at the individual and population levels, and to find the best means for the preservation of biotic communities which are in danger of destruction or transformation. In planning this programme it is intended in the first place to restrict it to clearly defined small projects of limited scope which can only be done by international co-operation. The work will be basic in character and although it will be co-ordinated with related activities of a more applied kind being carried out by such bodies as UNESCO, FAO, WHO, or IUCN, it will involve no duplication.

It is not surprising that after a period of such ferocious development the question has been raised whether the structure and mechanism of ICSU as laid down thirty years ago needs some revision. The great activity of the separate international unions may have concealed the importance of the national academies. Yet these latter have a vital part to play, especially to prevent a too extensive fragmentation of science, and through interdisciplinary integration to preserve science as a whole. They, too, by contact with Governments, are primarily involved in the search for funds. The whole structure must be designed to allow for this, and to ensure a satisfactory geographical distribution of members while still preserving both efficiency and flexibility. Also, one cannot escape the fact that this enormous growth of activity requires a larger central secretariat in the headquarters which are shortly to move from The Hague to Rome. Yet policy must always be decided by scientists themselves, and bureaucracy must be limited to the essential minimum. More money is needed. The Assembly agreed to double the subscriptions of National Members and to ask for additional voluntary contributions. UNESCO, an inter-Governmental organization, has certain overlapping interests with ICSU, but a mutually beneficial arrangement has been maintained, and UNESCO has continued to transfer funds to ICSU for use by the unions and special committees which are carrying out work in which it has a direct interest and on which it frequently seeks advice.

The ICSU Assembly has appointed a special committee to examine all these problems of structure and operation in the near future. Dr. E. W. R. Steacie, who succeeds Sir Rudolph Peters as president, will have charge of this committee. The next general assembly is planned for 1963 or 1964 in Zurich.

The spirit of ICSU to-day can still be expressed by the resolution of the 1946 assembly, also held in London, acknowledging the duty of scientific workers (*a*) to maintain a spirit of frankness, honesty, integrity and co-operation and to work for international understanding, (*b*) to promote the development of science in the way most beneficial to mankind and to exert their influence as far as possible to prevent its misuse, (*c*) to serve the community not only by their specialized work but also by assisting so far as they are able in the education of the public in the purposes and achievements of science. H. W. THOMPSON

OBITUARIES

Mr. B. B. Osmaston, C.I.E.

B. B. OSMASTON, known to many in India as "B. B.", was born at Yeldersley Hall, Derbyshire, in 1867, the ninth of a family of fifteen, two of whom survive him. He was educated at Cheltenham and at the Royal India Engineering College at Coopers Hill, where those entering the India Forest Service were then trained. He passed in and out first of his year at Coopers Hill, and was made a Fellow of the College. He joined the Forest Service in India in 1888, and served in the United Provinces (including Dehra Dun Forest College), Bengal, the Andaman Islands, a short time in Burma, and finally retired as chief conservator of the Central Provinces, being appointed C.I.E.

After retirement he lived with his family for some time at Srinagar in Kashmir, and for the rest of his life he lived at Oxford.

In 1892 he married Catherine Mary, daughter of General and Mrs. Hutchinson, and they had sixty-eight years of married life together. His wife died in 1960, but two sons and three daughters survive

him, one son having been killed in the First World War.

He was keen on any natural science subject which he took up: chemistry, botany, spiders and, above all, ornithology. He loved birds from boyhood, and he was very familiar with the habits and songs of Indian birds.

Mr. Osmaston's life in the jungle inevitably brought a large share of excitement and adventure in the form of encounters with dangerous wild animals, but one of the most exciting occurred within three months of his arrival in India, and is well worth recording again. He was posted to the district of Jaunsar in the hills, where there had been a man-eating tigress taking a toll of human life for ten years. Mr. Osmaston, then aged twenty-one, and a forest student named Mr. Hansard, decided to try to stalk the tigress in the heat of the day, when, they felt sure, she would be lying up in some secluded place. They went cautiously down a steep ravine which was covered with thick scrub, one of them on each side of the steep banks bordering the small stream. They had not been going for more than five minutes when Mr. Osmaston heard a scuffle and a terrible noise of growling, mixed with cries and groans above him. The tigress had stalked the stalkers, and had pounced on Mr. Hansard from behind. Mr. Osmaston, who had never before seen a tiger outside a zoo, had to risk a shot at the tigress while she was mauling Mr. Hansard. The shot made the tigress let go her hold, and the second barrel discharged at her as she came headlong down the bank towards him finished her off. Mr. Hansard's life was saved, though he had been badly mauled, and the scourge of the countryside was dead. Mr. Osmaston had achieved what many a famous hunter had tried to do during the previous ten years.

His adventures included an encounter on foot with the rogue elephant which had caused the death of Mrs. Anson; an exciting escape from drowning when shooting some rapids in a canoe; navigating a small launch by compass and map without previous experience, in order to find a certain uninhabited island in the Andamans which was reputed to be the only habitat of some very rare hornbills. This adventure included the onset of a storm resulting in the loss of the landing boat while the party was ashore, which necessitated a dangerous swim in rough water infested with sharks, back to the launch, which was anchored in deeper water.

These few incidents serve to show Mr. Osmaston's adventurous spirit. He was abstemious and thrifty all his life, and his disregard for personal comfort and safety took him to many strange and interesting places, and he had very wide knowledge of all things to do with life in the jungle.

After retirement he participated in two strenuous expeditions in pursuit of further knowledge about birds, the first to Ladakh at very high altitudes, and the second in Africa. Both these expeditions were undertaken in the company of his good friend Admiral Lynes, another keen ornithologist.

By his death, at the age of ninety-three, the world has lost a very courteous, charming and adventurous personality.

Dr. T. P. Colclough, C.B.E.

Tom Colclough, as he was known to ferrous metallurgists, who died on September 22, aged seventy-six, was one of those rare personalities who commanded respect in both industry and research. He was educated at the Universities of Manchester and Sheffield, and awarded the D.Sc. of Manchester and the D.Met. at Sheffield. He entered the Brown-Firth Research Laboratories in 1916 under W. H. Hatfield and in 1920 became chief chemist at Park Gate Iron and Steel Co., Ltd. He was appointed in turn chief metallurgist, open-hearth manager and technical officer. In 1929 he became technical director of H. A. Brassert and Co. and was directly concerned with subsequent developments at Corby, Ebbw Vale and other steel works. His capacity for sensing the direction of developments in iron and steel contributed to an international reputation covering Germany, Austria, Turkey, China and New Zealand.

During the Second World War he was technical adviser to the Iron and Steel Control of the Ministry of Supply, and was appointed C.B.E. for his services in 1947. In 1945 he continued in this field as technical adviser to the British Iron and Steel Federation, which post he held at the time of his death. His post-war travel ranged from work with the Control Commission in Germany at the end of the War, to advise the Durgapur works in India and the R.I.S.C.O. works in Southern Rhodesia. He was the leader of British Iron and Steel Federation missions to the U.S.S.R., Poland and Yugoslavia.

Research was not forgotten amid this industrial activity, for he participated in the work of the British Iron and Steel Research Association, and from 1950 served as a member of its Council. He was awarded the Bessemer Gold Medal of the Iron and Steel Institute in 1954, and elected an honorary vice-president of that Institute in May 1960.

Until recently he delivered an annual lecture in the Department of Metallurgy at Manchester, which was always received with eager concentration by the students. He had the power to hold the attention and stimulate his audience which scarcely changed with advancing years. In these days of specialization, his combination of forward thinking with the necessary restrictions of practice was as welcome as it was unusual.

C. R. Tottle

Dr. E. McKenzie-Taylor, C.I.E., M.B.E.

Dr. E. McKenzie-Taylor died at his home at Basildon on October 17. He was a North-country man and received his early training at Armstrong College, Newcastle upon Tyne, before the First World War; for a short time he was a demonstrator in agricultural chemistry there. His first appointment elsewhere was as lecturer in agricultural science at the Harper Adams Agricultural College. From there he moved to the East Anglian Institute of Agriculture at Chelmsford, where he became head of the Department of Agricultural Chemistry, and as such was concerned primarily with analytical work connected with agricultural problems.

After service in the Royal Artillery during the First World War, he returned to Chelmsford until 1921, when he was appointed senior chemist to the Cotton Research Board of the Egyptian Ministry of Agriculture. Here, with A. C. Burns, he carried out important work on the daily and seasonal changes in soil temperature and on other factors influencing the yield of cotton. In particular, they investigated the steady decrease which had been experienced in the yield of cotton in Egypt during the previous quarter

of a century, under intensive irrigation by free flow from the Nile.

McKenzie-Taylor returned to Great Britain in 1926 and was appointed University lecturer in agricultural chemistry in the School of Agriculture at Cambridge, a post which he held until 1930. In that year he became director of irrigation research and land reclamation in the Panjab. Here again he applied his active mind to the soil problems connected with irrigation. With his colleagues he was concerned to elucidate the mechanism of increasing salinity of the surface soil in irrigation areas, the possibility of the influence of a rising water-table on it, the difficulties of removing the accumulating salts, and the significance, in connexion with 'water duties', of using extra water to leach them out.

During the Second World War, McKenzie-Taylor became director of military engineering research, G.H.Q., India, where he held the rank of brigadier. After returning to England on retirement, he had, since 1952, acted as agronomist to Rotary Hoes, Ltd.

H. H. NICHOLSON

NEWS and VIEWS

Royal Society Awards : Royal Medals

H.M. THE QUEEN has approved recommendations made by the Council of the Royal Society for the award of the two Royal Medals for 1961 as follows: to Sir Wilfrid Le Gros Clark, Dr. Lee's professor of anatomy in the University of Oxford, in recognition of his outstanding contributions to neuroanatomy and primate morphology, which he has combined to provide new knowledge of human evolution; to Prof. C. F. Powell, Melville Wills professor of physics in the University of Bristol, in recognition of his pioneering work on the development of photographic emulsion technique in the investigation of cosmic rays and the outstanding results derived therefrom on the elementary particles in cosmic radiation.

Experimental Physics in the University of Sussex: Prof. K. F. Smith

DR. K. F. SMITH, at present lecturer in the Cavendish Laboratory, Physics Department, University of Cambridge, has been appointed to the chair of experimental physics in the new University of Sussex at Brighton. Prof. Smith was born in 1923 and went to Colfe's Grammar School at Lewisham (London). In 1942 he went to Cambridge with a minor scholarship to Sidney Sussex College. His studies were interrupted in 1945 by work on atomic energy, first in Canada and then at the Atomic Energy Research Establishment at Harwell. In 1948 he returned to Cambridge and began to build an apparatus for measuring nuclear spins, using the molecular-beam method, developed by O. Stern and further by I. I. Rabi, but not previously used in Great Britain. He aimed in particular at the study of unstable, that is, radioactive nuclei, of which only very minute quantities can be obtained. In subsequent years he and his students developed various ingenious ways of recording the faint trickle of atoms that pass through the severe scrutiny of narrow slits, two inhomogeneous magnetic fields and a radio frequency field which causes the spin of the atom to change direction. Research Fellow of Sidney Sussex College since 1950, Senior Exhibitioner in 1952, Dr. Smith became a demonstrator in the University of Cambridge in 1953, and lecturer in 1958. He spent a year's sabbatical leave in 1958 as an associate professor at Berkeley, California. He will be followed to Brighton by the good wishes of the molecular-beam fraternity everywhere.

Iodine-131 in Milk in Britain

RESULTS are now available for measurements, made by the Agricultural Research Council Radiobiological Laboratory, of iodine-131 in milk during the week ending November 11. The method of sampling was the same as that already described (see *Nature*, November 11, p. 504). Milk is collected daily from 23 sites in different parts of England, Wales, Scotland and Northern Ireland. In addition, on one day in each week country-wide samples are provided by more than 200 depots which together handle more than 40 per cent of the total milk produced throughout the United Kingdom. The mean results of the daily samples are shown in Table 1.

Table 1

Week	$\mu\mu$c. Iodine-131/litre
October 15–21	114
October 22–28	158
October 29–November 4	127
November 4–11	117

The survey of iodine-131 in milk throughout the United Kingdom has been in progress for 8 weeks and an analysis of the combined results of the daily and country-wide sampling now enables the overall situation to be assessed in terms of the recommendations made by the Medical Research Council. It was stated that the acceptable radiation dose would not be exceeded for infants under one year of age, the most susceptible age group of the population, unless the average concentration of iodine-131 in milk rose above 130 $\mu\mu$c./litre over a period of one year, or higher concentrations were maintained for correspondingly shorter periods. This recommendation took into account the likely extent of variations in the contamination of local milk supplies. The significance of the quantity of iodine-131 which has so far entered milk can thus be assessed by comparing it with that which would give rise to the annual average specified by the Medical Research Council. Table 2 shows that in different regions of the country, between 9 and 20 per cent of the specified annual total had been reached on November 11, the country-wide average being 14 per cent.

Table 2. SUMMARY OF RESULTS OF DAILY AND COUNTRY-WIDE SAMPLING OF MILK FOR IODINE-131 DURING EIGHT WEEKS, SEPTEMBER 16–NOVEMBER 11

Zone	Average contamination ($\mu\mu$c./litre)	Percentage of quantity which would give rise to annual average specified by the Medical Research Council
1. Northern England	103	12
2. North-West England	98	12
3. East and East Midland	72	9
4. West Midland	106	13
5. Wales	165	20
6. South and South-East England	94	11
7. Mid-Western England	100	12
8. Far-Western England	133	16
9. Scotland	157	19
10. Northern Ireland	164	19
Country-wide mean	115	14

The highest values have been found in Wales, Northern Ireland, Scotland and far-western England, but even these would not lead to the annual average specified by the Medical Research Council unless they continued for at least a further seven months or more. Regional differences can result not only from climatic and geographical factors but also from differences in the diet of cattle. The quantity of iodine-131 which they consume depends primarily on the quantity of fresh grass which they eat; thus, recently deposited fission products enter milk less readily when, owing to the onset of winter, cattle are provided with increasing quantities of other foods. In 1958, after the previous series of weapons trials in the Arctic, this effect was marked from the middle of November onwards, and results from Scotland suggest that it is occurring again. It cannot, however, be assumed that the contamination of milk will decrease steadily in the future, because for some weeks after the detonation of nuclear weapons the rate of deposition may vary widely depending on meteorological factors. A careful watch will therefore be maintained so long as appreciable levels of contamination remain.

Paul Instrument Fund Grants

Awards of grants by the Paul Instrument Fund Committee have been made as follows: £44,500, with the possibility of an increase to £52,000, to Dr. V. E. Cosslett, University lecturer in physics, University of Cambridge, to design and construct a very high voltage electron microscope and to explore its applications in biological and metallurgical research; £1,000, in supplement of a previous grant, to Prof. D. Gabor, professor of applied electron physics in the Imperial College, University of London, and Dr. D. Jones, for work on the development of an electron interference microscope; £3,500 to Mr. D. W. Hill, research department of anæsthetics, Royal College of Surgeons of England, for the construction of an infra-red gas or vapour analyser using interference filters; £2,500 to Dr. N. Kurti, reader in physics and senior research Fellow, Brasenose College, Oxford, for the construction of a nuclear thermometer for use in the micro-degree Kelvin temperature-range; £1,500, in supplement of a previous grant, to Dr. J. H. Sanders, University lecturer and demonstrator in physics, Clarendon Laboratory, Oxford, for the construction of an optical maser. The Paul Instrument Fund Committee, composed of representatives of the Royal Society, the Institute of Physics and the Physical Society and the Institution of Electrical Engineers, was set up in 1945 "to receive applications from British subjects who are research workers in Great Britain for grants for the design, construction and maintenance of novel, unusual or much improved types of physical instruments and apparatus for investigations in pure or applied physical science".

Department of Technical Co-operation

In a written answer in the House of Commons on November 14, the Secretary for Technical Co-operation stated that of the expenditure on overseas information services, 32 per cent was attributable to the British Council, 35 per cent to the British Broadcasting Corporation, 13 per cent to the Central Office of Information and 20 per cent to overseas departments. The Department had taken over 87 administrative and advisory staff from other Departments, including 31 from one of the Overseas Departments, 155 executive staff, and 306 ancillary staff. With four exceptions, the staff had been previously employed on work which had been transferred with them. He was now considering how the Department's ability to help with advice on the economic aspects of development could be strengthened.

Technical Colleges and Schools in Britain

In reply to a question in the House of Commons on November 16 regarding the number of technical colleges and schools in Great Britain, the Minister of Education, Sir David Eccles, said that in England and Wales there were 761 grant-aided major establishments, 232 maintained secondary technical schools and 5 direct-grant secondary technical schools. Students in the two latter numbered 97,039 and 838, respectively, in January 1961. In the 1959–60 sessions, 112,768 students attended full-time courses in the major establishments, 10,038 sandwich courses, 501,003 part-time and 784,584 evening courses. In the 92 major establishments in Scotland, there were 12,549 full-time students, 35,609 part-time and 52,696 evening students. Construction is in progress on 126 major projects in England and Wales and 18 in Scotland, and 217 further projects in England and Wales and 25 in Scotland have been approved.

High-flux Reactor at Petten

The high-flux materials testing reactor at the Petten research centre of the Reactor Centrum Nederland became critical for the first time on November 9. This is the second reactor to become critical at Petten since September 28, 1960, when a low-flux reactor was put into operation. The nuclear part of the high-flux reactor was designed and delivered by the U.S. firm of Allis-Chalmers Manufacturing Company, the remaining part having been built in co-operation with several Dutch companies. Most components, also those delivered by Allis-Chalmers, were manufactured by Dutch industries. The reactor, which is essentially similar to the U.S. Oak Ridge research reactor, is intended for the testing of materials. The neutron beams emitted by the reactor can also be used for carrying out neutron physics research and for the production of radio-isotopes. During the forthcoming months the reactor will be operated at low power. Afterwards the power will be gradually increased up to the maximum level of 20,000 kW. It is expected that this level will be reached in the spring of 1962. In the meantime, the reactor vessel, in which the actual core of the reactor is situated, will be replaced by a new one, which has been manufactured in the United Kingdom.

Digital Computation at King's College, Newcastle upon Tyne

A new automatic digital computer has been ordered by King's College, Newcastle upon Tyne, to replace the *Pegasus* computer in the Computing Laboratory. The new computing system is based on the *KDF.9* machine manufactured by the English Electric Company. A grant towards the cost of the machine has been promised by the University Grants Committee, while the balance remains to be found by the College. The new system is capable of very high speeds of calculation and has facilities for storing large quantities of information on magnetic tape. Results in both numerical and alphabetical form can be printed at speeds up to 600 lines per min. On some problems the new machine will operate at about one hundred times as fast as the existing machine, and will greatly assist many research projects in both pure and

applied science not only for the University but also for industry. It will permit also the training of many students in the techniques and applications of automatic computing. At the present time, computing systems are being ordered in increasing quantities for both scientific and commercial applications, and the machines are being built faster than people are being taught how to use them. There is thus great need for enlarged training facilities of the kind the Computing Laboratory can provide.

National Research Council of Canada

THE recently published forty-fourth annual report of the National Research Council of Canada outlines the scope of work carried out during 1960–61, including the provision of 7·1 million dollars to support pure research in the universities (including 775 grants, 550 scholarships and fellowships) and an additional 2·3 million dollars through the Medical Research Council (Pp. 48. N.R.C. 6322. Ottawa: Queen's Printer, 1961). The National Research Council employs 670 scientific research staff (including 130 postdoctorate fellows) and 953 personnel, and operates five laboratory divisions covering the fields of applied biology, applied chemistry, pure chemistry, applied physics and pure physics; and also controls the four engineering divisions, including building research, mechanical engineering, the National Aeronautical Establishment, and radio and electrical engineering as well as two regional laboratories, one at Halifax and the other in Saskatoon. During 1960–61 it sponsored thirty-six associate committees, operating in such diverse fields as the control of hospital infections, experimental psychology, plant breeding, radio science, and soil and snow mechanics; and dealt with 14,500 technical inquiries from Canadian industries. The outstanding event of the year was the establishment of the Medical Research Council as an autonomous body associated with the National Research Council. The report also includes the annual reports of the Medical Research Council and Canadian Patents and Development, Ltd.

The *Review of the National Research Council*, Canada, for 1961 (Pp. 377. NRC. No. 6421. Ottawa: National Research Council, 1961. 75 cents), describes the work of the Council to March 31, 1961, and besides reports of the directors of divisions and heads of sections includes accounts of the work of the Council's committees and the first review of the work of the Medical Research Council, 1960–61. Lists of publications during the period are appended to the several reports of divisions, etc., and with the directory of staff and list of staff serving on outside organizations the *Review* is a comprehensive reference work on the two Research Councils, supplementing the information given in the annual report for the period and in the report on university support.

Safety in Mines Research

THE annual report of the Safety in Mines Research Establishment of the Ministry of Power for 1960 gives a concise yet easily readable account of the wide range of work undertaken by the Establishment (London: H.M.S.O. Pp. 72 + 4 plates. 5*s*. net). Within the main body of the report sections are devoted to explosives and blasting devices, explosion hazard, fire hazard, rescue apparatus, engineering and metallurgical research, dust measurement and the pneumoconiosis hazard, investigation of mining incidents, examination of equipment after mine service and testing services. Special attention is being paid in the field of coal dust explosions to the use of stone-dust barriers and a full-scale explosion gallery is to be built at the Buxton laboratories. Most coal dust explosions underground are initiated by the ignition of firedamp; accordingly, the work on layering of firedamp and its accumulation has been extended together with an examination of preventive and remedial measures. At the same time research on the frictional ignition of gas, particularly between metals and rock, is in progress. Ignitions of gas due to shot-firing are very few compared with the tens of millions fired each year; but such ignitions are always serious; present work in this field is concerned with the safety of millisecond-delay firing and with improved methods of testing explosives. Much of the work described in the report will be of interest to many fields of industry other than the coal industry not only in connexion with explosion and fire hazards but also in connexion with breathing apparatus, engineering and metallurgical research, dust measurement and pneumoconiosis.

Wool Research Colloquia in Australia

A NEW series of one-day meetings has been inaugurated in Australia to discuss particular aspects of wool research. They are being organized by the Commonwealth Scientific and Industrial Research Organization Wool Research Laboratories, and invitations are extended to those research workers in university and C.S.I.R.O. laboratories who are working on the subject under consideration. Wool Research Colloquium No. 1, which was held at the Division of Protein Chemistry, C.S.I.R.O., Parkville, Victoria, on September 20, was convened by Dr. R. D. B. Fraser. Wool structure was considered at this meeting under the headings of molecular structure, chemical structure, biosynthesis and physical properties and structure. Wool research scientists from several States of the Commonwealth attended the meeting. Prof. H. Fraenkel-Conrat, of the Virus Laboratory, University of California, who had been in Melbourne as guest lecturer during a series of lectures in protein chemistry, and Mr. I. K. Walker, director of the Dominion Chemical Laboratory, Wellington, New Zealand, were guests at the Colloquium. These meetings will be a permanent feature and they are intended to provide specialist discussions on major aspects of the C.S.I.R.O. wool research programme.

Research and Education in Physics

AN international seminar for research and education in physics, co-sponsored by the International Atomic Energy Agency, the United Nations Educational, Scientific and Cultural Organization and the Central Committee for Swedish Technical Assistance, is to be held as a one-year course at the Institute of Physics, Uppsala, during September 1, 1962–July 1, 1963. The aim of the seminar is essentially to provide possibilities for individual participation in qualified experimental research work in one of various fields of physics (for example, solid-state physics, X-ray physics, nuclear physics, atomic physics, etc.) under the guidance of experienced scientists. An introductory course in the operation of, and the coding for, a modern computer, IBM 1620, will be given. The seminar will be open to non-European students and scientists, mainly from developing countries, who are working in the above-mentioned subjects and who are connected with the teaching and/or research of a university or national

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laboratory, and who are not less than twenty years and not more than forty years of age. Application forms and further information can be obtained from the International Seminar, Institute of Physics, University of Uppsala, Uppsala, Sweden.

University News :

Belfast

The following appointments to lectureships have been announced: Dr. R. H. J. Sellin (civil engineering); F. J. Smith (applied digital mathematics computing); Dr. Uno Opic (applied mathematics).

Bristol

The following appointments to lectureships have been announced: C. Andrew (mechanical engineering); Dr. R. Binks (organic chemistry); J. S. E. David (veterinary surgery—diseases of reproduction); Dr. R. P. Mercier (theoretical physics); Dr. J. G. C. Milne (physics); D. B. Peacock (bacteriology).

Cambridge

The following elections at Churchill College have been made: *Fellowships*, J. A. Pateman, University lecturer in genetics, and C. K. Phillips, deputy treasurer of the University; *Junior Research Fellowship*, J. S. Gibson, of the University of Sheffield, in genetics; *Extraordinary Fellowship*, Prof. Jacques Barzun, professor of history, Faculty of Political Science, provost of Columbia University for 1962–63, and Prof. Kenneth J. Arrow, professor of economics and statistics, Stanford University, for 1963–64; *Overseas Fellowship*, Dr. P. W. Anderson, of the Bell Telephone Laboratories.

Ibadan

The Calendar 1961–62 of the University College, Ibadan, Nigeria, lists the officers, standing committees, principal standing boards and committees of the Senate, honorary associates and the staff of the College (Pp. 110. Ibadan: The University College, 1961). Besides particulars of entrance regulations, courses of study, scholarships and prizes, there are notes on the Department of Extra-Mural Studies, Institute of Education, Institute of Child Health, and the Nigerian Institute of Social and Economic Research. A list of graduates for 1961 is also included.

London

Prof. K. G. Denbigh, professor of chemical engineering at the Imperial College of Science and Technology, has been appointed to the Courtaulds chair of chemical engineering tenable at that College. The following titles have been conferred: professor of geography, on Dr. W. R. Mead in respect of his post at University College; professor of organic chemistry, on Dr. L. N. Owen in respect of his post at the Imperial College of Science and Technology; reader in anatomy, on Mr. D. W. James in respect of his post at University College; reader in chemistry, on Dr. C. A. Vernon in respect of his post at University College.

Miami

Dr. Carl H. Oppenheimer, formerly of the University of Texas Marine Institute at Port Aransas, has been appointed research associate professor at the Institute of Marine Science, University of Miami, where he is at present head of the microbiology group.

Announcements

Prof. J. B. S. Haldane, formerly professor of biometry in the University of London and later research professor in the Indian Statistical Institute, from which post he has recently resigned, was awarded this year the Kimber Gold Medal for genetics of the U.S. National Academy of Sciences. He has also received a Premio Feltrinelli for biological sciences of the Accademia dei Lincei, Rome. Prof. Haldane attended a special meeting during the summer of the Physiological Society held in Oxford to commemorate the centenary of the birth of his father, J. S. Haldane, and received an honorary doctorate of science from the University. He expects to take charge of a small laboratory for research in genetics to be set up by the Council for Scientific and Industrial Research, India.

Dr. Leo L. Beranek, president of Bolt Beranek and Newman, Inc., of Cambridge, Mass., has been presented with the Wallace Clement Sabine Award of the Acoustical Society of America for his "internationally recognized achievements in all phases of architectural acoustics". The Pioneers of Underwater Sound Award has been presented to Dr. Albert B. Wood, of the Admiralty Research Laboratory in Teddington, Middlesex, "for nearly half a century of original work in physics and for decades of pioneering leadership in underwater sound".

Dr. F. Ralph Kotter has been appointed chief of the newly established High Voltage Section of the Electricity Division at the National Bureau of Standards, U.S. Department of Commerce. Since 1955, Dr. Kotter has been working on standards of inductance and capacitance at the Bureau.

Dr. Thomas R. Lawrence has been appointed to the National Bureau of Standards, U.S. Department of Commerce. As a member of the Engineering Metrology Section, Dr. Lawrence will investigate the applicability of the helium–neon gaseous optical maser to precision measurements of length.

The Systematics Association is organizing a lecture on "The Systematics of Man", to be delivered by Dr. B. G. Campbell at the British Museum (Natural History) on February 8. Further information can be obtained from Mr. Norman Tebble, Systematics Association, British Museum (Natural History), Cromwell Road London, S.W.7.

An international symposium on the growth and development of the lower archegoniate plants will be held in the rooms of the Linnean Society of London, Burlington House, Piccadilly, London, W.1, during April 5–6, 1962. Further information can be obtained from the General Secretary of the Society.

Errata. In the article entitled "The Mechanism of Action of Liver Alcohol Dehydrogenase" in *Nature* of October 7, p. 47, the following corrections should be made: Table 1: $\varphi_{12} = 0{\cdot}9$ sec. μM^2; $\varphi_{12}' = 10{,}500$ sec. μM^2; $K_{E\text{-alc}} = 6060\ \mu M$; in Fig. 1: 2·2 μM LADH in (3), (6) and (7); col. 1, line 5 from bottom of p. 49, ($pK = 8{\cdot}6$).

Dr. D. B. E. Stroud, writing in connexion with the article "Mannosyl-D-Glucosamine Hydrochloride Isomers" (*Nature*, **191**, 142; 1961), states that the optical rotation $[\alpha]_D^{20} + 55°$ quoted on p. 143, three lines from foot of col. 2, refers to the original mixture of fractions $A + B$, and not to fraction B.

WATER POLLUTION RESEARCH IN GREAT BRITAIN

A FURTHER stage in the development of the Water Pollution Research Laboratory of the Department of Scientific and Industrial Research was marked by two open days at Stevenage on October 11–12, when interested members of the public could see a wide range of work in progress and also inspect the new wing which came into use during the summer.

The new west wing, designed by the Ministry of Works, provides an additional floor space of nearly 20,000 sq. ft. and comprises four pilot-scale laboratories, with several smaller laboratories and offices (Fig. 1). One of the pilot-scale laboratories rises through three storeys and the others are two storeys in height, giving adequate headroom for treatment equipment of all kinds. The construction is in load-bearing brickwork, which facilitates the fixing and support of apparatus. A full range of services is provided, and the keynote is flexibility.

The fact that this new wing has become necessary —it is already almost fully occupied—only seven years after the Laboratory moved to Stevenage from its temporary headquarters at Watford is a sign of the increasing need for research into all aspects of the pollution of water. Three recent Acts, the Clean Rivers (Estuaries and Tidal Waters) Act, 1960, the Rivers (Prevention of Pollution) Act, 1961, and the Public Health Act, 1961, have extended control over the discharge of wastes into rivers and estuaries, and the field of pollution prevention and control is now a very active one, in which local authorities, river boards and industry are all closely concerned.

In order that the work of the Laboratory can be more closely applied in Scotland, an out-station has recently been established at East Kilbride, near Glasgow. This station carries out its own local research and can also call on the resources of the main laboratory at Stevenage when necessary.

The functions of the Water Pollution Research Laboratory are, broadly, to study and improve methods of treating polluting liquids, including both domestic sewage and trade effluents, and to study the effects of pollution on surface waters. The scope and variety of the work involved are so great that only a few examples can be described. Two subjects of wide general interest may be mentioned first.

The survey of the Thames Estuary, which was started in 1948, is now nearing completion. Its primary object was to provide information for the guidance of the Pippard Committee, the report of which is now with the Minister of Housing and Local Government and is expected to be published shortly. Analytical records collected by the London County Council during some sixty years, supplemented by detailed surveys made by the Laboratory staff, have been used to study the complex relationships between dissolved oxygen, temperature, pollutional load, freshwater flow and other factors. A theory of mixing developed at the Laboratory has enabled predictions to be made of the effect of changes in the pollutional load on the condition of the estuary water. It has given good results when applied to changes known to have taken place in the past. Fresh problems have presented themselves as the survey has proceeded. For example, the role of nitrates as a reserve of oxygen, the rate of nitrification of polluting matter, and the effect of synthetic detergents on the rate of settling of suspended solids have all required special study before the complete picture of conditions in the estuary can be built up.

The second subject of general interest is the search for synthetic detergents which can be broken down biologically in sewage treatment works and will not produce the familiar and objectionable foam on rivers. During recent years the Laboratory has tested 74 new materials for manufacturers on a repayment basis. It has also collaborated in the 'Luton experiment', in which a 'soft', straight-chain detergent, sodium 'Dobane *JN*' sulphonate, has, since 1958, been distributed by the manufacturers in Luton and the surrounding district instead of the normal 'hard' detergent. An extensive programme of sampling at Luton sewage treatment works and in the River Lea has shown a reduction of about 40 per cent in the surface-active material entering the river. This is rather less than expected on the basis of laboratory experiments, a result which may be due to the unusual method of treatment used at Luton, consisting of high-rate activated sludge treatment followed by high-rate biological filtration. A greater reduction of the new material has been observed at some other sewage works in the area, and this disparity between different methods of treatment is being further examined in the Laboratory.

Fig. 1. The Water Pollution Research Laboratory. Pilot-scale laboratories in the new west wing

Treatment of Sewage and Industrial Wastes

Little information has hitherto been available on the relative merits of the various filter media used in the biological filtration of sewage. Since April 1959 a portion of the Stevenage domestic sewage has been treated on a battery of eight small filters filled with two different sizes (nominally about 1 in. and 2½ in.) of four different media (clinker, blast-furnace slag, broken rock and rounded gravel). With the exception of the gravel which, in the smaller size, gave some ponding trouble during last winter, the smaller media have consistently produced better effluents than the larger media. The media with the larger surface area per unit bulk volume, namely, the clinker and slag, gave better results than those of more regular shape, namely, the broken rock and rounded gravel. These relative efficiencies of treatment applied not only to the removal of biochemical oxygen demand but also to the reduction in bacterial counts.

Investigations into the activated sludge process have been principally concerned with the efficiencies of various methods of aeration. The work has suggested ways in which the efficiencies (on a basis of oxygen put into solution per unit of power supplied) accepted in normal sewage treatment practice could be considerably improved by such means as altering the geometry of the aeration tanks. Whether the gain in efficiency would be outweighed by other disadvantages such as increased capital cost would depend on local conditions.

Interesting fundamental studies have been started on the kinetics of the activated sludge process, to determine the relative importance of the method and intensity of aeration, the level of dissolved oxygen and the concentration of sludge maintained in the tanks, the degree of turbulence and longitudinal mixing and other variables.

There is an increasing demand for investigations into methods of pre-treating specific industrial wastes, and this work is done by the Laboratory on repayment. Recent and current work of this nature includes the removal of ferrocyanide from engineering wastes, the electrolytic oxidation of cyanide, the bacterial oxidation of thiocyanates, the use of beds of calcined magnesite to neutralize acid wastes, and the treatment of washings from cattle sheds and other farm premises—this last being a growing problem which sometimes causes difficulty at small sewage works.

River Pollution

A large part of the work on rivers is devoted to assessing the relative effects of surface aeration, photosynthesis and plant respiration on the oxygen balance. As a positive step towards increasing the oxygen content of a polluted river, experiments are being performed on air entrainment in a Venturi tube, which could be inserted in an effluent pipe near the point of discharge to a river. The method is simple and, on the laboratory scale, has shown promising results.

Work on the toxicity to fish of ammonia and other chemicals has continued, and the effects of mixtures of toxic substances are now being examined.

Measurement Techniques

It is inevitable that a laboratory such as this should from time to time find that existing standard methods of analysis and measurement are unsuited to its needs in many specialized lines. A valuable part of its work is therefore the improvement of old methods and the development of new. Among its contributions in this way are the improvement of established methods for determining nitrites and nitrates, the introduction of new techniques for assessing organic carbon and for the separation of radioactive strontium and barium, and the design of automatic sampling equipment for sewage, and recorders for dissolved oxygen and suspended solids.

The work of the Water Pollution Research Laboratory is thus seen to be a judicious combination of fundamental investigation and practical application for the benefit of the man on the job. On the impressive evidence put forward during the open days, it seems to be doing this work very well indeed.

F. E. Bruce

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION, AUSTRALIA

THE thirteenth annual report of the Commonwealth of Australia Scientific and Industrial Research Organization for the year ended June 30, 1961, besides being a general review and a brief survey of some of the more important developments arising during the year out of the research being carried out by the Organization, includes a list of papers published during the year and details of the membership of the Advisory Council, the State Committees and staff*. Total expenditure amounted to £9,603,963 and the Treasury appropriation to £7,571,104, of which £1,058,246 was in the animal research laboratories, £886,518 on plant research, £334,613 on food preservation, £396,600 on forest products, £856,192 on the chemical research laboratories, £246,683 on fisheries and oceanography, £767,705 on the National Standards Laboratory, £451,278 on radio physics, £574,252 on wool research, £280,904 on fuel research, £333,237 on land research and regional survey, £51,700 on research association grants and £95,069 on overseas research studentships. While since 1950 the total budget increased from £2·7 million to £9·6 million, the research staff only increased from 750 to 880, and the Executive believes that this increase in staff is quite insufficient.

The giant radio telescope being erected at Parkes for the Division of Radiophysics is structurally completed and is in fact now available full time for research. The Controlled Environment Research Laboratory is scheduled for completion early in 1962.

Among the research developments noted in the review are a new method of stimulating the germination of legume seeds by exposing water-soaked seeds

* Commonwealth of Australia. Thirteenth Annual Report of the Commonwealth Scientific and Industrial Research Organization for the year ending June 30, 1961. Pp. iii + 150. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1961.)

to low concentrations of carbon dioxide; a practical and economic means of controlling posthitis in wethers by implanting under the skin small pellets of the male hormone, testosterone; progress in work on worm parasites of sheep and cattle; and evidence that selection of cattle for their resistance to ticks may be a highly satisfactory means of minimizing losses due to tick infestation. Codling moth infestations have been found to depend largely on the larvæ which over-winter in an orchard, and these, in turn, on the amount of cocoon shelter available in the orchard. This can be reduced by adequate management, and, if trap bands are provided to which the overwintering individuals are attracted in large numbers and destroyed by special toxic deposits, relatively few insecticidal treatments are needed in the following season. Work at Katherine has shown that nitrogen in a form available to plants concentrates just under the soil surface during the dry season and the greatest loss occurs during the wet season, owing to leaching by rain.

It has now been demonstrated that when the appropriate fertilizer dressings to overcome deficiences in nitrogen, phosphorus, potassium, sulphur, calcium, zinc and molybdenum are applied to the infertile coastal lowlands of southern Queensland, pastures can be grown on these soils which grow and fatten cattle at stocking-rates of a beast to 1·5 acres, with annual live-weight increases of 300 lb. per beast. Investigations on the role of elements in plants have shown that manganese deficiency inhibits a reaction involved in the evolution of oxygen during photosynthesis and also that manganese is a constituent of normal chloroplasts. Studies on the life-habits of the freshwater snail *Lymnea tomentosa*, which infests grazing lands, have revealed great difficulties in applying effective control measures, as if the population is drastically reduced each surviving snail will harbour so many liver flukes that the end result is much the same. Spoilage of dried prunes of high-moisture content has been prevented by hot-filling the prunes into laminate bags.

A direct-reading tester for increasing the regain of wool has been developed, consisting of a hot-air blower to dry the wool and a balance which indicates regain directly. Evidence has been obtained that substantial numbers of freezing nuclei occur at altitudes of 50,000–60,000 ft., and work has been completed on a recorder, designed to operate for periods up to 12 months, to provide continuous records of water-level in streams, dams or bores for gauging purposes, or of rainfall, temperature, humidity, wind speed or evaporation. A process has been devised for production of pure thorium by converting thorium carbide to volatile thorium iodide and depositing thorium metal from this on to a heated surface. A new cyclone elutriator, the 'Cyclosizer', developed for precise separation of solid particles in the subsieve ranges, is based on the stable flow patterns and high centrifugal forces obtainable in hydraulic cyclones of suitable dimensions.

A thorough examination has been made of resistance measurements to establish clearly the possible limits of accuracy using both direct and low-frequency alternating current; and fundamental investigations of cell wall organization of the tissue elements of wood and of the pits between them has demonstrated that in hardwoods the initial penetration path is through the vessels and then via the pits to the rays and adjacent fibres or vertical parenchyma. In softwoods the path is from tracheid to tracheid, or tracheid to parenchyma, through the pits. A notable advance in the metal-staining technique for the electronmicroscopy of wool has revealed further structural details within the microfibrils and a resolution of 1 in 4,000 has been achieved with a mass spectrometer constructed at the Chemical Research Laboratory, while new techniques, involving an optical filter of narrow band-width, have provided photographs showing the velocity distribution in selected areas of the solar chromosphere.

SCIENTIFIC RESEARCH IN NEW ZEALAND

THE report of the Department of Scientific and Industrial Research, New Zealand, for the year ended March 31, 1961*, includes the Minister's Statement, the report of the Council of Scientific and Industrial Research and that of the Secretary, while the third annual report of the Ross Dependency Research Committee is appended. Lists of publications during the year are given under the respective branches of the Department together with lists of projects in hand.

Expenditure during the year amounted to £1,963,486 net, of which £201,421 was on the Dominion Laboratory, £225,114 on the Dominion Physical Laboratory, £131,777 on the Grasslands Division, £124,885 on the Geophysics Division, £114,532 on the Geological Survey and £111,986 on the Soil Bureau. The Council again points out that New Zealand is still lagging behind in the application of science and the scale of research, and her expenditure in 1959–60 of about £3·98 million on scientific work or 0·32 per cent of the gross national produc compares with 2·3 per cent in Britain and 2·7 pe cent in the United States in 1957–58. Professional staff at April 1 was 401 compared with 390 in 1959–60, total staff being 1,121 and 1,067, respectively, and the Secretary refers to a loss of 30 professional staff during the year, overseas, nearly all experienced scientists, although the overall calibre of the Department's staff has substantially improved over the past decade. During the past five years M.Sc. graduates in physics in New Zealand universities have averaged 10, in chemistry 21 and in mathematics 13, but about half those obtaining first-class honours accept posts overseas and do not return. The Secretary stresses the importance of being able to attract scientists of high quality and indicates some of the factors, besides adequate salary, which are important. In 1960 the establishment of two senior research fellowships tenable in the Department's laboratories for up to two years was approved and two fellowships have now been awarded. The Council is gravely concerned, however, that no action has been taken towards securing the 40–50 increase in staff per annum

* New Zealand: Report of the Department of Scientific and Industrial Research for the year ended 31 March, 1961. (H. 34.) Pp. 87. (Wellington: Government Printer, 1961.)

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over the next five years which is required to implement fully the Department's present programme of work.

After discussions with the University Research Committee and the chairman of the new University Grants Committee the Council proposes to terminate the present type of research grant made to the universities by the Department. From the end of the present financial year the Department will sponsor and provide funds for research units in universities in fields of study deemed essential in the national interest, and in which an outstanding man is stationed in the university, and by entering into research contracts with universities. Grants to research and allied institutions in 1960–61 amounted to £176,862, of which £33,810 was to universities and agricultural colleges and £91,376 to research associations.

The Council's recommendation for the establishment of a new Wool Research Organization as an incorporated research association with an annual income of £100,000, half from the New Zealand Wool Board and half from the Government has been approved, and the new buildings for the Meat Research Institute were completed during the year. The Council has decided to continue New Zealand's scientific programme in Antarctica at about its present level (£86,606 in 1960–61), and topographical and geological survey work will be continued until the reconnaissance of the Cape Adare–Beardmore section is complete. Some concern is expressed as to the effect of delay in developing the Institute of Nuclear Sciences on the research programme in this field.

Points in the research work completed during the year noted in the Secretary's report include the completion of the first volume of the new *Flora of New Zealand*; the high yields now being obtained with Aotea wheat, bred at the Crop Research Division and released in 1957; the marked difference in the distribution of radioactive selenium in plants; the variation of the nitrate-levels and of sodium, potassium, calcium, manganese, aluminium, copper, titanium, iron, zinc, sulphate and phosphate in ryegrass plants, and the study of the systematics and taxonomy of bacterial plant pathogens.

The Fruit Research Division's apple-variety collection has led to the selection of the Canadian variety Spartan and a local seedling, Red Fresca, for extensive commercial trial, and satisfactory drying of tobacco leaf has been obtained by two methods which enable a kiln to be loaded with much less labour and permit the use of smaller kilns. A study has been made of the Adelie penguins breeding at Cape Royds, Ross Island; and a set of 54 soils selected representing a range of New Zealand soils for agricultural, forestry and highway purposes is being examined comprehensively by chemical physical and biological tests.

An intensive mineralogical study of volcanic ash showers has been commenced, and the four-mile geological map project commenced in 1956 to be completed in 1964 has already indicated areas meriting more detailed examination. A rugged tester, in which the sample is dried for 5 min. in hot air and weighed on scales calibrated directly in water content (corrected for buoyancy) has been developed by the Dominion Laboratory, and 20 of these machines are now in use in scouring works and freezing works throughout New Zealand for controlling the drying of wool. A fœtal heart-rate recorder has been constructed using a heart-sound pick-up which delivers this conglomeration of sounds to a system specially designed to deal with a fœtal pulse which may be mostly even below the level of background noise. Investigations of the variation in the essential oil derived from samples of *Pinus radiata* have been initiated, and an instrument developed for rapidly and accurately determining the density of a wood sample taken from a living tree, utilizing a very narrow beam of beta-radiation from a strontium-90 source. A novel type of navigation light for marking narrow channels has been developed which can be used when the configuration of the land in line with the channel will not permit the use of more than one light. An emergency wheat-testing service provided by the Wheat Research Institute avoided substantial loss during the 1961 wheat harvest. A survey of currents in McCurdo Sound established that at least in summer there is a definite movement of water towards the Antarctic Ice Shelf.

FOOT-AND-MOUTH DISEASE

IN a report issued by the Research Institute (Animal Virus Diseases), Pirbright, Surrey*, the director, Dr. I. A. Galloway, describes the investigations made into foot-and-mouth and related virus diseases over the past five years.

The research programme of the Institute is considered in relation to foot-and-mouth disease in Britain, and, in turn, as a world problem. In Britain, periods of relative freedom from the disease (or at least of low incidence) are interrupted by times when as many as 200 farms may become infected in one month. These periods of higher incidence do not generally last more than three or four months, and the number of animals involved in relation to the animal population of the country remains less than half of 1 per cent. It is generally agreed that a vaccination programme against the disease in a country where it is endemic is unlikely to reduce the incidence much below this figure. If, therefore, the disease were allowed to become endemic in Britain, and vaccination were decided on, the ultimate goal which could reasonably be achieved would be an incidence equivalent to that which now obtains. At that point, a slaughter policy would undoubtedly be invoked to attempt to achieve eradication of the disease. If the situation in the European countries is considered, it is seen that, in spite of an increasing extension of vaccination since the 1951–52 epidemic, the incidence of the disease, though greatly reduced, is still somewhat higher than in Britain. Some countries are applying, and others considering, limited slaughter of infected animals as a further step towards eradicating the disease. Outside Europe, in endemic areas, effective control of the disease is still some way off, and it is here perhaps that the new improved vaccines have the greatest part to play, if the cost of the vaccination campaign can be reduced and the efficiency of the vaccine increased. Assistance in reduction of the incidence of the disease elsewhere

* Research Institute (Animal Virus Diseases), Pirbright. Report for the years 1956–1960. Pp. 52. (Pirbright, Surrey: Research Institute (Animal Virus Diseases), 1961.) 2*s*. 6*d*.

than in Britain is at present the greatest contribution which can be made towards the control of the disease within the country.

A considerable proportion of the research effort at Pirbright has always been directed at improved methods of immunization against the disease. The period under review has seen a new bias on work on immunization. The development of live modified strain vaccines received a new impetus by the demonstration by Skinner in 1951 of the possibility of infecting unweaned mice and, in 1954, of passaging virus in day-old chicks and chick embryos. Serial transfers, repeated sometimes many hundreds of times, have the effect of reducing the capacity of the virus to produce visible signs of the disease in the cattle inoculated. By multiplying in the animal, however, the virus would stimulate the defence mechanism of the host and so produce a solid long-lasting immunity. Such vaccines are used in many other diseases; for example, in rinderpest, rabies, African horse sickness, blue tongue, and in yellow fever and also in smallpox in man. Workers developing such strains aim at the point at which the virus will 'infect' the animal but produce no clinical signs. The yellow fever vaccine inoculated in man is an example of a live virus vaccine which has reached this point. With most live vaccines, the ideal is not attained, and a compromise between slight infectivity in the sense of producing the clinical disease on one hand, and a capability which falls short of the maximum in producing immunity on the other, has to be accepted. This has been true in developing the strains in foot-and-mouth disease and the problem has been made more difficult by the demonstration that not all strains become attenuated or lose virulence at the same rate. On the other hand, there has been little indication of difficulty in some other ways; for example, there has been no restoration of virulence and no spread to normal animals in contact with those vaccinated. Limited experiments on pregnant animals have indicated no unfortunate sequelæ, and young animals have been successfully vaccinated.

The variation in behaviour of viruses has made the development of suitable strains for each of the seven types of virus now known a complicated operation, especially when several different strains of one type have had to be developed to explore the possibilities of different methods of attenuation. Nevertheless, strains of six of the seven types have reached the point at which tests for non-infectivity in cattle have been sufficiently encouraging to warrant investigations on the immunity produced. These tests at Pirbright have now involved approximately 2,000 animals.

The qualified successes obtained under experimental conditions at Pirbright led to the next phase in development of these strains. Since the step from the laboratory to field conditions might not be without risk, it was considered that first trials outside the laboratory should be conducted in areas where the disease was endemic. The Institute has had the willing and enthusiastic co-operation of the Governments of countries in South and East Africa in a series of trials. These experiments have indicated the potential usefulness of suitable strains in such animal populations while, at the same time, bringing to light many of the suspected difficulties inseparable from immunization of a mixed population with possible previous experience of the disease. Some of these experiments, notably those in South Africa, have involved tests of live attenuated vaccine on a scale which has not been undertaken with any other agent of this type, in respect of severe challenge with fully virulent material. Even under these conditions, 70 per cent protection was obtained and subsequent use of the vaccine in an infected area resulted in an incidence of less than 1 per cent on vaccinated farms, while, on unvaccinated farms, the incidence was between 60 and 70 per cent. These observations are essentially preliminary; many problems require investigation.

The problem of the use of inactivated vaccines has not been neglected. Frenkel-type vaccine in which virus is grown in culture of cattle tongue epithelium, adsorbed on aluminium hydroxide and inactivated with formalin and mild heat, has been a useful agent and will continue to play a most important part in the control of the disease as it has done in Europe and, to some extent, in South America. Extensive tests of this type of vaccine have been carried out at Pirbright and several large batches have been prepared and tested for safety and potency. Other possible sources of tissue in which virus may be grown, such as kidney, are being investigated, since kidneys present a more easily obtainable source of tissue than cattle tongue epithelium.

Foot-and-mouth disease must be considered as a world problem. Part of the work at Pirbright, therefore, has been directed towards the assessment of the world situation, particularly in regard to the prevalence of the disease and the distribution of the immunological types of virus. Earlier reports have referred to the discovery at Pirbright of four new types—*S.A.T.* 1, *S.A.T.* 2 and *S.A.T.* 3 from Africa, and Asia 1 in many countries in Asia. In the past twelve years considerable progress has been made in determining the broad outlines of type distribution. The international importance of this work was recognized when, in 1958, the Institute was nominated by the Food and Agriculture Organization of the United Nations to undertake the functions of World Reference Laboratory for Foot-and-Mouth Disease.

APPLIED GEOGRAPHY

THE application of geography to the study of a wide range of problems has made remarkable progress in recent years, partly during, and partly because of, the Second World War. Geography made a substantial contribution to the British War effort both in the planning of campaigns and in the reorganization of the nation's economy, and since the War geographers have been active in the field of planning. Some of the fruits of these efforts can now be seen in the increase in the number of geographers who act as advisers in various fields, ranging from problems of conservation to aspects of medical geography: there is also the evidence of the results achieved by those who have been largely employed in the study of

the application of geography in Britain to national, regional and county affairs during recent years.

At the recent meeting of the British Association for the Advancement of Science a whole morning was devoted, largely by the geographers of Section E, to the planning problems of Britain and to those of East Anglia in particular. The future administrative organization of Norfolk and Suffolk is at the moment under review by the Boundary Commission, and during the course of the meeting geographers saw, in the field, the significance of the desire of the rapidly growing and increasingly industrialized city of Norwich to expand into its adjoining county areas. Others were shown the changing landscape of Thetford, where residential and industrial developments are designed to absorb part of the overspill of Greater London.

Among the speakers in the planning symposium were Mr. J. R. James, deputy chief planner, Ministry of Housing and Local Government, and the principal research officer of the Ministry, Dr. E. C. Willatts, who introduced a new map, at the scale of 10 miles to the inch, showing the major post-war developments in England and Wales that have resulted from, or are affecting, land planning[1]. These developments, including the establishment of National Parks, the building of electrical and nuclear power stations, the construction of motorways, the creation of 'new towns' and the extension of other towns, are indicative of both the scale and scope of post-war changes. They show, too, how the regional geography of Britain is in process of being transformed by the technological revolution which, in its geographical aspects, was the subject of Prof. S. H. Beaver's presidential address to Section E[2].

In the siting of many of these new developments geographers have sometimes played a significant part. An assessment of the scope of geographical inquiry and of the fields in which geographers are capable of making important contributions was made by Prof. L. Dudley Stamp in a small book, published in 1960, *Applied Geography*[3]. Such a book could have had no better author than Prof. Stamp in view of his own great efforts in many of the fields of which he writes: these activities have included the Land Utilisation Survey of Britain, carried through under his direction during the 'thirties, a particularly difficult and trying period for British agriculture[4]; the investigations of the Scott Committee, of which he was vice-chairman, into the use of rural land in Great Britain[5], and his later work as an adviser to the Minister of Agriculture on these problems; his initiative in establishing an organization for a World Land Use Survey under the auspices of the International Geographical Union[6]; and his work on the geographical background of many new developments oversea both within and outside the tropics.

Some of Prof. Stamp's topics have been further discussed by other geographers in the recently issued proceedings of the Anglo-Polish seminar held in Nieborów, Poland, in September 1959, and attended by twelve British and seventeen Polish geographers. The volume, *Problems of Applied Geography*, has been published in Warsaw by the Institute of Geography of the Polish Academy of Sciences, which organized the seminar in collaboration with the Institute of British Geographers, with the generous support of Unesco and of the British Council: limited supplies of the volume are available for sale in Britain[7]. The seminar, like that organized a year later for the exchange of views between American and Polish geographers[8], included a series of papers—six British and five Polish—which served as a basis for discussions between the delegations and was followed by a comprehensive tour of both agricultural and industrial areas of Poland.

While most of the six British papers have nothing strikingly original about them, they form a valuable and up-to-date summary of the special problems of Britain to-day as seen by a group of geographers. As with the Polish delegation, the British team emphasized the achievements and experience of the theory and practice of planning in its own country, while suggesting that many of its conclusions are of use to all concerned in the application of geography to the study of physical, social and economic problems in contemporary society. M. J. Wise's paper outlines the basic problems of national planning in Britain, including the optimum use of resources, the redevelopment of the older industrial areas, the restriction of the growth of conurbations, and the regulation of population movements; and A. A. L. Caesar shows how these problems vary between regions. H. C. K. Henderson describes the basis of the pre-war Land Utilisation Survey of Britain and indicates how its work has been used in the planning of agricultural land use. S. H. Beaver describes the reclamation of industrial waste-land for agricultural and other purposes, illustrating his paper by a series of carefully chosen examples: he shows that the area of land in Britain that cannot be reclaimed—less than 1,700 acres a year—is minute compared with the loss of agricultural land to urban development. It is this continuing urban encroachment on the countryside that A. E. Smailes in his paper on perhaps the most distinctive feature of British economy and society—the urbanization of Britain—sees as the crux of the physical planning problem of Britain to-day. Finally, K. C. Edwards, who, as president of the Institute of British Geographers, was leader of the delegation, discusses the 'new towns' of Britain, the results of which, he finds, though "modest in scale and often despairingly slow in realization, at least encouraging".

The Polish contributions have special value because so little information is easily available about modern Poland, certainly in English. They have been deliberately designed to match the British papers: thus the importance in Poland of regional planning, land use and urban geography are discussed by K. Dziewoński, J. Kostrowicki and L. Kosiński respectively. The two other Polish essays are of a very different type, describing the preparation of a geomorphological and hydrographic map and an investigation of local climate: their inclusion in the symposium suggests that there is in Poland a greater appreciation of the significance of such detailed and specialized studies in practical affairs than has so far been the case in Great Britain, where research of this nature would, to most administrators and planners, seem not only academic but even irrelevant.

But what will strike readers of this volume most forcibly will be the fundamental contrasts between the British and Polish attitudes towards planning and between the nature and scope of the planning envisaged for the two countries. In Britain the problems are those of adjustment and reconciliation rather than those of development—the transformation of the population pattern and economic organization developed in one industrial age to what is required in another, and greatly different, age of new and rapidly advancing technology. Poland is concerned with the fundamental development problems that call for comprehensive and large-scale

regional planning as a basis for the reconstruction and expansion of the national economy; and Dziewoński's paper shows that the methods and results of geographical research in Poland have exerted very considerable influence on the methods and results of planning. For this there is not, and probably never will be, any exact British parallel, but Polish experience does heavily underline the diversity of the ways in which the work of geographers can be used for the nation's good, if they are given the opportunity and the encouragement.

International seminars of this type broaden the minds of those participating and at the same time help to create deeper understanding and friendship, while the circulation of the report of the proceedings will be of lasting value to all who are interested in applied geography and who are concerned that it should have the closest possible connexion with life and society.

ROBERT W. STEEL

[1] The map prepared by the Ministry of Housing and Local Government is being published by H.M. Stationery Office.

[2] "Technology and Geography", to appear in *The Advancement of Science*, December 1961; and summary in *Nature*, **191**, 986 (1961).

[3] Stamp, L. Dudley, *Applied Geography* (1960).

[4] See especially Stamp, L. Dudley, *The Land of Britain: Its Use and Misuse* (1948).

[5] *Report of the Committee on Land Utilisation in Rural Areas* (Cmd. 6378) (H.M.S.O., London, 1942).

[6] Four of the World Land Use Survey's reports have so far been published, the most recent being Niddrie, D. L., *Land Use and Population in Tobago* (Geographical Publications, Bude, 1961).

[7] *Problems of Applied Geography*, Geographical Studies No. 25, Polish Academy of Sciences, Institute of Geography (Warszawa, 1961). Pp. 148, with photographs, maps and diagrams. Copies (price 25*s*.) can be obtained from the treasurer of the Institute of British Geographers, Dr. H. C. K. Henderson, Birkbeck College, Malet Street, London, W.C.1.

[8] See Hartshorne, R., "The American–Polish Geographical Seminar and Excursion, 1960", *Geog. Rev.*, **51**, 443 (1961).

GAS CHROMATOGRAPHY IN MEDICAL RESEARCH

THE Gas Chromatography Discussion Group of the Institute of Petroleum held an informal symposium on September 26, at the Royal College of Surgeons of England in London.

In welcoming the members of the Group, Prof. R. F. Woolmer, on behalf of the President and Council, explained that, apart from surgery, the College research and teaching also covered such associated fields as physiology, biochemistry, dental science and anæsthetics. It was appropriate that a symposium concerned mainly with the aspects of gas chromatography in medical research should be held at the College, as they believed in co-operation and a full exchange of ideas with other workers.

In the opening paper, Dr. A. T. James described the development and operation of a simple detector for the continuous automatic determination of the radioactivity of carbon-14 and tritium. Attention had been directed to automatic determination because of the obvious disadvantages of the previously used discontinuous methods. Problems of stability at column temperatures as high as 200° C. had led to combustion of the eluted material to carbon dioxide and water, and the measurement of the radioactivity at room temperature. The detector consisted of a 4 × ½ in. copper tube, plated internally and having a central tungsten wire electrode. Rhodium plating reduced the tailing exhibited by tritiated materials. Argon or helium was used as carrier gas, and carbon dioxide was bled into the feed to the detector to give a concentration of approximately 5 per cent. Chromatograms obtained with conventional detectors and the radioactivity detector were shown to superimpose extremely well. An integrated record could also be produced.

The next paper, on gas-liquid radiochromatography, by Dr. S. Popják, confirmed the disadvantages of discontinuous procedures for radioactivity measurements. The advantages of continuous monitoring over fraction collection had been evident over a period of three years using a scintillation counter. In the method described, carrier gas and eluted fractions were passed through a liquid scintillator in a specially designed apparatus, and the radioactivity measured by scintillation counting. Two isotopes could be measured simultaneously. Full details of the construction of the apparatus, its method of operation and the associated electronic circuitry were given. A stepped record superimposed well on chromatograms obtained with a gas density balance. The efficiency of counting was of the order of 40–50 per cent for carbon-14 compounds, and 15–21 per cent for tritiated compounds.

Under the title of "Peak Symmetry in Gas-Solid Chromatography", Mr. C. G. Scott described recent work concerned with the properties of two active solid systems giving symmetrical elution peaks for hydrocarbons at column temperatures below the boiling point of the eluted component. When alumina is modified with sodium hydroxide, the energy of adsorption decreases to a constant value, at which stage symmetrical elution peaks are obtained. Such peaks can also be obtained from columns packed with solid organic crystals, although, to increase surface area and so reduce column lengths, the compounds are best deposited on 'Celite'. It had been found that heats of adsorption were of the same numerical magnitude as the heats of vaporization. Entropies of adsorption were calculated for *n*-heptane, benzene, iso-octane and *cyclo*hexane, and values obtained for the first two compared with those obtained by Kemball for adsorption on mercury.

Mr. D. W. Hill and Dr. R. P. W. Scott described recent developments in the design of compact cathode-ray tube gas chromatographs. Apart from the high speed of analysis for vapours containing anæsthetics, the apparatus should be compact for easy transport and use in an operating theatre, and be capable of giving rapid repetitive results. The instrument was designed around a 6-in. square, long-persistence tube having a 20–30 sec. scan, and contained its own pump. An additional port in the sample injection system allowed a 100 : 1 gas dilution without the high losses of argon given by 'splitter systems'. The micro argon detector had been modified to reduce the effects of electron capture so that the strongly electron-capturing halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) gave a positive peak. Column packings for the separation of typical anæsthetic mixtures were mentioned. The quantitative response of the argon detector was dependent on temperature, and therefore the use of a thermostat or compensation was necessary. A simple thermistor compensator unit was described.

XUM

Dr. R. A. Butler presented a paper entitled "Parts Per Million and All That" in which he first described his work on anæsthetics, indicating the importance of knowing the concentration of anæsthetics in tissue, the rate of its elimination from the tissue, and equilibrium values of the various substances used. He queried the accuracy of calibration standards made from volatile substances, and consequently the accuracy of a detector calibration using such standards. He felt that some absolute figures often quoted in papers were at the least rather optimistic, and his paper was obviously designed to stimulate a discussion on the quantitative aspects of gas chromatography. It was unfortunate and disappointing that it failed to do so.

A contribution by Dr. T. R. Phillips described how, during the analysis of Arcton 12 (dichlorodifluoromethane) in Arcton 11 (monochlorotrifluoromethane), the calibration of the apparatus had to be carried out with synthetic mixtures of the substances and the samples taken at a fixed pressure. The sensitivity of the apparatus for Arcton 12 expressed as peak-height/partial pressure was shown to vary with the total pressure of the sample and with the partial pressure of Arcton 11. It was thought that this effect could occur in other systems where a small amount of a weakly retained substance was being analysed in the presence of excess of a more strongly retained one. Dr. Phillips's suggestion that the effect would not be observed if integration of the peak were used instead of the measurement of peak height was confirmed by other contributors to the discussion.

The concluding paper on electron capture was given by Mr. N. L. Gregory. He described a detector to which a potential of only 10–20 V. was applied and which utilized recombination effects to measure compounds having an affinity for free electrons. It was extremely sensitive to halogenated compounds, a typical application being to the detection and determination of insecticides in foodstuffs. Another application was to the determination of tetraethyl lead in gasoline, there being no response to the hydrocarbons present. Apart from halogenated compounds, polycyclic aromatic hydrocarbons, unsaturated fatty-acid esters and quinones were also good electron acceptors. The possibility of the affinity of organic compounds for free electrons in biochemical processes was mentioned.

Members of the Group were also privileged to view apparatus, watch demonstrations, and discuss gas chromatographic techniques with workers in various departments of the College, and also in the adjoining Nuffield College of Surgical Sciences. This was much appreciated by those present.

C. E. H. Knapman

INDUSTRIAL TRAINING IN EUROPE

Speakers from Belgium, Holland, Sweden and Switzerland joined colleagues from Britain at the annual conference of the British Association for Commerical and Industrial Education which was held at the University of Nottingham during September 14–16, when an examination was made of education and training on the continent of Europe, with particular reference to the effects of economic integration. The theme of the conference is of special significance since free movement of labour among Common Market countries may lead to standardization of systems of education and training, or at least a levelling up of standards.

One common thread ran through all the papers —the need to direct more attention to general education in modern schemes of training. All the countries present are now beginning to examine their training systems from this point of view.

Among the contributors were Monsieur R. Grandbois, inspector-general of the French Ministry of Education, who stated that all differences concerning apprenticeships are a consequence of the past. They are the result of local habits and customs ; they represent the embodiment of a way of life, the outcome of an evolution both social and administrative. All the similarities are either a consequence of the present or a result of the pressure of things to come. The various systems of apprenticeship have evolved slowly to their present-day form and crystallized behind innumerable frontiers and in confined surroundings, but they are now and will henceforth be subjected to influences for which barriers and remoteness do not exist. It can be assumed that the apprentices of to-morrow will have to be educated in much the same way whatever the country to which they belong.

Monsieur G. C. M. Hardebeck, secretary of the Bemetal Foundation in Holland, believes that more attention must be directed to general education because it is our responsibility not only to give young people the necessary technical training but also to prepare them to live in a democracy.

In his paper, Mr. H. A. Warren, principal of the South-East London Technical College, explained how in Great Britain at a time in the 1930's, when thousands of man-hours were being idled away in unemployment, the young apprentice was asked to work 48 hours a week and in addition, three evenings at college and two more on homework. A few million pounds extra expenditure on day release at that time would have meant that Britain to-day would no longer be suffering from a lack of skilled man-power. Warren suggested that the wholly evening course, except for recreation or for advanced refresher course studies, is as out of date as the steam locomotive ; despite this, Britain has the administrative arrangements which, to meet the challenge of the changing nature of skill, stand out as far superior to anything in continental Europe. "Nowhere in Western Europe is the sandwich course at the C.A.T. [college of advanced technology] level and standard growing to full and recognized stature as it is in Britain".

The pattern of industrial education might have been greatly strengthened had the City and Guilds of London Institute been entrusted also with the General Certificate course at least up to the Ordinary Level of the General Certificate of Education.

The British system is weak because of its lack of incentive. Since no end-of-apprenticeship tests are insisted on, the studies for apprentices in a technical college are voluntary both in the sense of attendance, and in the sense of acquiring a qualification. When, in addition, little recognition will accrue in wages or promotion, the student not unreasonably asks himself why he is pursuing his studies.

Prof. G. Dunand of the International Labour Organization showed how economic mutual penetration has been practised in Europe on a minor scale for centuries. For such exchanges it was accepted and convenient to use mainly one language, Latin, and later French, and more recently English, and also German in Eastern Europe. The universal use of French or English for European relations has, however, been increasingly questioned since language has become an essential element of conscious nationhood and indeed nationalism. Nowadays, supremacy would not readily be granted to the language of any one of the great powers, as, for example, English or Russian. Hence the multiplication, in particular since 1919, of bilingual treaties and multi-lingual international conferences, which have given birth to the profession of conference interpreter and to an extraordinary development of translation for international relations.

From this historical review, Dr. O. G. Pickard, principal of the Ealing Technical College and School of Art posed the question: "Do we then ask the schools to devote less attention to French and take up other languages in its place ?" This has been tried; during the interim period of the two World Wars there was a determined attempt to introduce a great deal of Spanish to English schools for commercial reasons in place of French. The immediate result of this attempt was to antagonize the teachers of French all over Britain, who clearly saw their vested interest in the teaching of this language threatened by the proposals to introduce Spanish in its place.

So far as the schools are concerned, the question is "whether a third foreign language should find a place in the school curriculum so that it might be possible for school children to learn say, French, German and Russian, or French, Spanish and Swedish, as a normal practice during their school careers. With the growing close association between the economy of Britain and that of Western Europe, it may be necessary to do much more language teaching in schools than has been the practice hitherto [in Britain]. This in turn may lead to a lengthening of the secondary school course as happened on the Continent where it is unusual for the secondary school course for the grammar school stream to finish before 19 or 20 years of age". Neither the schools nor the Ministry of Education have taken kindly to this particular view. The place for teaching the less common languages may be in further education.

Dr. M. B. Brodie of the Administrative Staff College, Henley, and Dr. Walter Goldberg of the Gothenburg School of Business Administration, Sweden, were both concerned with management development. Brodie stated that businesses which expand and grow more complex in character require relatively more managers. While quantitative estimates must be cautious, since they are likely to transpose present-day notions and assumptions too readily to the future, one estimate which attempts to allow for qualitative changes suggests an increase of about 50 per cent in the number of executives needed over the next ten years.

Throughout Europe, the shortage of staff of calibre for teaching and for research in managment is widespread and serious. Weakness on the staff side cannot but jeopardize the whole quality and character of any programme. "The limitations of educational ventures run with second-rate staff are too manifold to need enumerating."

Dr. Goldberg gave details of important Swedish developments in the field of management education. In 1957, for example, an investigation showed that the relative number of academically trained top-level managers since 1944 had increased from 58·5 to 84·5 per cent in industry, and from 43 to 54·5 per cent in other enterprises. Sweden proposes to increase its intake of students in business schools to 700 in 1970—more than double the 1955 figures of 325.

All the papers have been brought together in a publication, *Continental Comparisons*, which can be obtained from the British Association for Commercial and Industrial Education, Publications Department, 26*a* Buckingham Palace Road, London, S.W.1, price 10*s*.

PERUVIAN ORCHIDS

THE appearance of Part 4 of Charles Schweinfurth's *Orchids of Peru** marks the completion of a task which has necessitated continuous and concentrated research over a period of many years. At last we now have a comprehensive modern account of the orchids of an Andine country, a region which is not only one of the richest in orchids in the world, but which is the original home of the parents of many of our finest cultivated orchid hybrids. This excellent account will not only be of service to students or visitors wishing to identify Peruvian orchids but will also facilitate the study of orchids from the neighbouring Andine countries of Bolivia, Ecuador and Colombia which have many species in common with Peru.

This final part contains among others the important genera *Odontoglossum*, *Miltonia* and *Oncidium* (including *Cyrtochilum*), the last-named being represented by more than 70 species. Like the three preceding parts, Part 4 is well illustrated, containing thirty-three charming line drawings and one half-tone plate of representatives of all the more important genera, thus bringing the number of illustrations in the whole work to 194. Of special interest are the three pictures of the remarkable alpine genus *Telipogon* most members of which are found only above 2,000 m. altitude.

Closer examination of the text in all these parts shows, however, how much more collecting is necessary in Peru before a really satisfactory final account of its orchid flora can be prepared. The author has in many cases not seen any specimens of the species dealt with, having had to be satisfied with descriptions or photographs of the original specimens scattered in various herbaria. Here then is an evident field for future work, since it cannot be doubted that with really good field collections available many of the outstanding problems could be solved and a more complete account given even of those species which are comparatively well known. V. S. SUMMERHAYES

* Chicago Natural History Museum. *Fieldiana: Botany*. Vol. 30, No. 4: *Orchids of Peru*. By Charles Schweinfurth. Pp. v+787–1005. (Chicago, Ill.: Chicago Natural History Museum, 1961.) 4.50 dollars.

XUM

AGE OF THE BASALT FLOW AT OLDUVAI, EAST AFRICA

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AND
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THE site of Olduvai in Tanganyika, East Africa, is known all over the world because of its remains of early man, the sequence of human industries and rich associated fauna, and we are all greatly impressed by the research, performed under difficult conditions, by Dr. and Mrs. Leakey. Four different beds have been distinguished. Bed I is underlain by a massive flow of basalt, and it should be possible to obtain here an age determination by means of the potassium–argon method. We are greatly indebted to Mr. S. E. Ellis of the Mineralogical Department of the British Museum (Natural History), who kindly gave us a rock specimen for that purpose (B.M. 1947/211: collected by K. P. Oakley). This sample is called here *O* I. As many implements at Olduvai are executed in basalt, we used two pebble tools from Bed I (collected by v. Koenigswald, 1951), in order to try to get information for Bed I ; these are the samples *O* II and *O* III.

Table 1. Potassium–Argon Ages of Basalt Samples from Olduvai
$\lambda_\beta = 4{\cdot}74 \times 10^{-10}\ a^{-1}$; $\lambda_k = 0{\cdot}58 \times 10^{-10}\ a^{-1}$

Sample	Weight (gm.)	A^r_{40} (10^{-7} c.c./gm.)	A^{at}_{40} (per cent)	K (per cent)	Age with potassium–argon ($10^6\ a$)
O I	0·52	0·58	88·6		
	0·51	0·60	87·9	1·13	1·3 ± 0·1
	0·51	0·57	90·6		
O II	0·51	1·91	70·2		
	0·71	1·93	69·6	2·17	2·25 ± 0·16
	0·51	1·94	72·4		
O III	0·52	0·93	89·8		
	0·60	0·86	89·3	1·67	1·4 ± 0·1
	0·61	0·96	88·0		
	0·69	0·92	87·9		

If we try to establish the age of Bed I with the help of the fauna, we find that *Elephas planifrons* and *Stegodon*, typical for the Villafranchian of Africa, are missing. There are many archaic types still present such as *Dinotherium* and *Mastodon*, but the dominant species among the elephants is *Elephas recki*, representing the same evolutionary stage as *Elephas antiquus* in Europe. Beds I and II have many species in common which do not occur higher up. According to Hopwood in ref. 1: "There is no faunistic evidence to suggest that the lower part of the Olduvai series is of Lower Pleistocene age". Leakey expressed the opinion that Olduvai "extends from the beginning of the Mindel to the very end of the Riss glaciation" (ref. 1, p. 160). After the discovery of *Zinjanthropus*, however, Leakey included Bed I in the Lower Pleistocene, the Villafranchian. Considering the various views expressed without further discussion we expect that Bed I has an age of 0·4–0·8 m.yr. (ref. 2) and 0·1–0·8 m.yr. (ref. 3) respectively.

The basalt bed, however, has a considerably higher age determined by using potassium–argon. The analysis has been carried out on stony material reduced to small pieces. The average diameter of the fragments of the rocks was about 0·8 mm., which guarantees sufficient homogeneity.

The basalt below Bed I has an age (determined using potassium–argon) of about 1·3 m.yr. The pebble tools from Bed I are not younger but older, and therefore not suitable for our purpose. The age of *O* III, however, is only a little higher than that for *O* I and might be regarded as confirming the figures for *O* I.

We see that the age determined with potassium–argon is about 0·5 m.yr. higher than we had expected them to be. There are three possibilities for this: (*a*) The age determined with potassium–argon is too high, because the amount of argon-40 (rad) is accumulated by inherited argon. This cause can only be excluded by further work on distant samples. (*b*) The sedimentation at Olduvai did not start directly after the formation of the basalt flow but considerably later. (*c*) Bed I has an age of about 1·0 m.yr. and the former estimates were not correct.

It is not easy to say which of the three alternatives may give us the right solution. Adopting the last suggestion we would be forced to revise considerably our ideas about the duration of the Pleistocene, which might be necessary, but should only be done if more age determinations of Pliocene and Villafranchian rocks are available. Therefore, it seems likely to us for the time being that a time-interval does exist between the basalt and the formation of Bed I.

Just after Gentner and Lippolt had finished their report on these measurements, an article was published by Leakey *et al.*[4] on the age of Bed I at Olduvai. Their figures, based on biotite, oligoclase and anorthoclase crystals from various tuffaceous intercalation are higher (1·6–1·9 m.yr.) than ours, though their samples are geologically younger. We thus wonder where this discrepancy comes from.

We can exclude that this is conditioned by the technique of measuring, since we are in good agreement with the age determination of a late Trinil basalt from Java measured by Evernden *et al.* Both laboratories obtained an age of 0·5 m.yr. for this sample, which may be used to date the *Pithecanthropus erectus*.

Thus the difficulty comes from the sample itself. In connexion with another problem, we investigated different Pleistocene samples (biotite and sanidine) originating from tuffs and found that their ages, determined with potassium–argon, do not necessarily have to agree with the geological sequence, but may be too high because of inherited argon. There is every appearance that this error may be present just in tuff minerals of low ages.

Furthermore, we have some sedimentological and palæontological questions concerning an extended time-scale.

For the top of Bed I the average age is given as 1·23 m.yr., for the lower part as 1·75 m.yr. Consequently, the total of Bed I should have been formed during a period of (at least) 0·50 m.yr. One might expect that such a long period should be subdivided by fauna, sedimentation, dry and wet periods.

industry; nothing of that kind has been observed. Bed I is about 30 m. thick, which would mean an average rate of sedimentation as low as 0·06 mm. per year. Where are the sediments to support this? By such a slow rate of sedimentation every bone would completely disintegrate before it would have a chance to survive as a fossil, but Bed I contains a rich fauna. Certain places might have been temporarily dry, to allow occupation sites, but no soil horizons have been formed or marked disconformities. Does not the preservation of the bones and the general unity of fauna and industry through the whole of Bed I give the impression of a 'normal' sedimentation?

Perhaps there is a marked time-interval between Bed I and Bed II. If we look at this new time scale, the top of Bed I is estimated to be 1·23 m.yr. old. According to the same authors a post-Chellean II tuff in Bed II has yielded a date of 0·36 m.yr. If we estimate the bottom of Bed II to be 0·50 m.yr. old, the supported interval would last nearly 0·75 m.yr. Here again we might ask why the faunæ of Beds I and II are roughly the same, having in common even archaic types of elephants, and why both beds, possessing the same grey colour, seem to rest conformably on each other. Where are the soils or zones of weathering which would certainly have been formed during this considerable interval of time and should separate the beds?

As the lower part of Bed I is said to have an age of 1·75 m.yr. the whole Pleistocene Period would have lasted well over 2·00 m.yr. This is more than double of what we, cautiously and with good reasons, have estimated.

Having considered the many unanswered sedimentological and palæontological questions which make Olduvai a difficult test case, we prefer, as stated here, to be cautious until more dates for the lower Pleistocene are available.

[1] Leakey, L. S. B., *Olduvai Gorge* (Cambridge Univ. Press, 1951).
[2] Zeuner, F. E., *The Pleistocene Period* (Hutchinson, 1959).
[3] Emiliani C., Mayeda, T., and Selli, R., *Bull. Geol. Soc. Amer.*, **72**, 5, 679 (1961).
[4] Leakey, L. S. B., Evernden, J. F., and Curtis, G. H., *Nature*, **191**, 479 (1961).

THROMBOCYTOSIS EVOKED BY EXERCISE

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AND

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THE mechanism(s) responsible for the rapid, over-all increase in hæmostatic activity in association with physical, emotional and other stress situations[1–4] has not been ascertained[4]. Admittedly, the plasma fibrinogen content has been said to be elevated in such circumstances[4], but judging from Selye's survey[2] this response generally takes several days to reach maximum. This is in accordance with later work by Rizza[5], who found that acute exercise in human subjects does not raise the plasma-levels of fibrinogen, prothrombin, Christmas factor or Factor V. However, in the two normal subjects examined, Rizza observed some increase in the activity of the antihæmophilic globulin and was inclined to believe that the hypercoagulability associated with exercise might possibly be traced to this change. Similar increase in the activity of the antihæmophilic globulin has been found after adrenaline infusions[6]. On the other hand, it has been mentioned that the platelet count is also increased in stressful situations[4], but according to the evidence given by Selye[2] this response is not wholly consistent. While moderate, short-term exercise has been found to raise the platelet count[7,8], it appears that following prolonged[9,10] as well as brief, strenuous exercise[11] no appreciable changes occur in the platelet count. Gerheim and Miller[11] expressed the opinion that "the greatly increased circulation velocity during exercise may destroy enough platelets to mask a moderate increase in numbers".

It seems possible, then, that the hypercoagulability associated with exercise and related conditions is ascribable to thrombocytosis rather than to any increase in the levels of the coagulation factors in the plasma. At all events, however, the question remains what is the ultimate mechanism of the thrombocytosis that seems to prevail in stressful situations. It has been conjectured that the leucocytosis known to accompany exercise and related conditions is effected mainly by the concomitant increase in circulation[12–14]. Conversely, the reversible hypothermic leucopænia (associated with thrombocytopænia and bleeding tendencies) has been traced to the reversibly retarded circulation inherent in hypothermia[15]. To our knowledge, nevertheless, no attempts have been made to substantiate or quantitate the possible role of the hæmodynamic changes in the establishment of these corpuscular blood changes. Hence, we have examined the relevant hæmatological changes in human subjects undergoing graded muscular exercise as well as the relation of the changes to those in cardiac output and allied parameters.

The counts of erythrocytes, leucocytes and thrombocytes (Fonio's method; counted per 2,000 red cells), including the hæmatocrit readings, were determined from the capillary blood and, in a part of the tests, also from the venous blood. For work-loading, a bicycle ergometer and marching were employed. The cardiac output was determined by using the dye-dilution technique, and from the dilution curves mean circulation time was reckoned. From the circulation time, again, 'relative circulation velocity' was derived (inverse value of the circulation time).

The results obtained, with special reference to the platelet and circulatory changes, may be outlined as follows.

In the eight subjects studied, light muscular exercise lasting for some 10 min. and capable of raising the cardiac output by 50–200 per cent resulted in an appreciable and consistent rise of the platelet count in the capillary blood. The figures obtained before and immediately after the exercise were 123,000–243,000 per mm.^{3} and 168,000–270,000 per mm.^{3}, respectively. The percentage increments were 9–37, and the greatest numerical increment recorded amounted to 58,000 per mm.^{3}. The platelet count in the venous blood showed a comparable increment, even if the figures were somewhat higher throughout. The 'relative circulation velocity', derived from the mean circulation time, was in all cases increased in

direct proportion to the increments in the cardiac output. Of special interest is the observation that the increments in the platelet count and in these two circulatory parameters were fairly commensurate.

More strenuous exercise of about 18 min. duration (testing the physical working capacity by Sjöstrand's method) in the same subjects yielded a more pronounced increase of the platelet count in the capillary blood; the resting values were 138,000–291,000 per mm.³, whereas after the work-load was imposed the figures were 210,000–348,000 per mm.³. The highest numerical increment was 107,000 per mm.³, and the percentage increments varied from 17 to 60. The recovery studies performed in this group of tests revealed that after 15 min. rest the platelet count had actually returned to the pre-exercise level (113,000–261,000 per mm.³).

Ten miles marching within some 90 min. resulted in considerably different changes. In the ten subjects studied there were no marked alterations in the platelet count in the capillary blood; in eight individuals the platelet count showed increments of 2–22 per cent, whereas in two instances the count tended to decrease (4 and 8 per cent). After a 90-min. recovery period the platelet count remained substantially unaltered in most instances. Yet, in three cases it was found to be increased no matter what the response to the work-load was.

In this connexion it may be mentioned that the changes in the leucocyte count after these different work-loads and recovery periods were the same in direction as, but usually relatively greater than, those in the platelet count. There was a good correlation between the increments in the leucocyte count and those in the changes in circulation examined. The hæmatocrit values usually rose but only slightly; the greatest increase encountered was 5·5 vol. per cent. The small degree of hæmoconcentration indicates that the increased counts of platelets and leucocytes cannot be accounted for by the concurrent hæmoconcentration.

These preliminary results suggested the tentative conclusion that the leucocytosis and even thrombocytosis that accompany at least light and moderate short-term exercise are actually effected by the concomitantly accelerated circulation. The source of the leucocytes and platelets which appear to be released into the circulation may be the pulmonary vascular bed, particularly its venous side, which is known to remove and discharge leucocytes[14,16] and platelets[13] on changing circulatory conditions. But also, similar release may occur in the peripheral vascular compartments in general, as suggested by the biomicroscopic observations on the microcirculation in hypothermic subjects (cf. Sarajas[15]). Moreover, since the work of Eberth and Schimmelbusch, it is known that on acceleration of circulation leucocytes and platelets enter into the axial flow in increasing numbers (cf. Koller[17]). Especially during prolonged and brief, strenuous exercise other factors, such as an altered fragility and adhesiveness of these cellular blood elements, may come into play, and this would explain the slight and inconsistent leucocyte and platelet changes encountered in our marching tests.

The clear-cut and consistent rise in the platelet count, as observed in the present tests with light and moderate exercise of short duration, makes it evident that the hypercoagulability in association with exercise[1–3] is possibly related to a thrombocytosis and that this thrombocytosis is occasioned by the concomitant circulatory activation. The same mechanisms may be responsible also for the hypercoagulability said to accompany other stressful situations[2–4].

Finally, the question arises whether the thrombocytosis and hypercoagulability of this origin operates particularly in the establishment of coronary thrombosis, which is known to be precipitated by states of emotional and physical stress.

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MODIFIED CUVETTES FOR PHOTOELECTRIC SPECTROPHOTOMETRY

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With the development of photoelectric spectroscopy to the point where even colorimetric techniques come closer and closer to being true spectrophotometry, the conventional vessel in which most solution measurements are made, and to which most optical coefficients are referred, has become the optical cuvette with 10 mm. light path. It is the purpose of this article to report a new approach to the problem of making spectral observations over an extended wave-length range, with convenience and accuracy, using modified cuvettes of this type. The discussion is centred around the use of hæmoprotein solutions, for the analyses of which this technique was evolved. It is stressed, however, that the principle is of the widest application and may be used over the whole of the spectrum wherever solutions are to be examined, whenever the solution would normally have to be diluted at some stage of the spectral examination.

It is often possible to arrange to dilute a solution so that it gives a conveniently measurable optical density over the whole of the wave-length range to be studied. There are occasions when this is not feasible, however. For example, many hæmoproteins (other than cytochromes) have relative extinctions at the ultra-violet, near ultra-violet, visible, and near infra-

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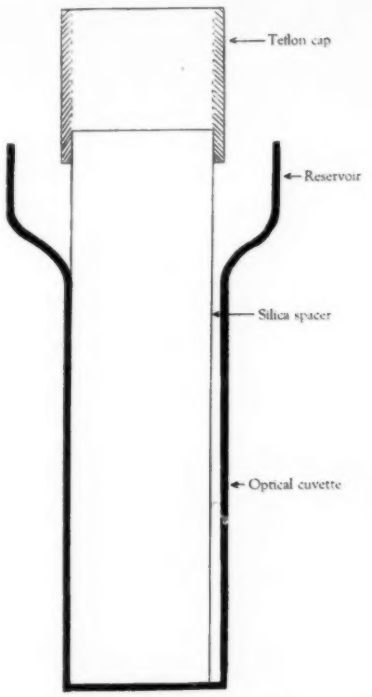

Fig. 1. General purpose cell. Optical path in plane of paper

red maxima of roughly 30, 100, 10 and 1; a range which is too great for the spectrophotometer to cover with accuracy, and certainly not with suitable clarity in the overall spectrum. The desired result can only be obtained by starting with a solution which is of sufficient strength to resolve adequately the lowest peaks of the spectrum, and then reducing the optical density in order to resolve peaks which would otherwise be too intense. This may be done in only one of two ways: by diluting the solution (assuming that it still obeys Beer's law), or by reducing the optical path of the original solution.

Dilution, as a routine answer, may be criticized on numerous grounds: Beer's law may not be obeyed by the solution; the process of dilution is time-consuming and inconvenient; mere dilution may upset equilibria in the experimental solution so that a special dilution medium may have to be prepared; dilution may in itself be undesirable where only small volumes of material are available to start with. There remains the possibility of a mechanical reduction of the optical path of the starting solution. Three methods are available:

(1) *The use of an adjustable cell.* These are expensive, need relatively large volumes of solution as compared with the standard cuvette, and must be provided with a reservoir to take the overflow when the path-length is reduced. In addition, they are difficult to rinse adequately; and once rinsed they are difficult to dry adequately.

(2) *The use of a second cell with a shorter path-length, for example, a 1-mm. cell.* This is certainly much more satisfactory than (1). There are still disadvantages, however. In the first place, with cells as narrow as 1 mm., capillary effects make emptying and filling the cell rather a problem. This means using a hypodermic syringe to get the solution in and out of the cell. This is reasonably satisfactory, but the very fact that a second cell is employed more than doubles the actual manual operations involved.

(3) *The use of a block of suitable material, for example, silica, inserted into the cell.* In this case, the cell has to be partly emptied before inserting the spacer or else there is an overflow. Furthermore, the amount of fluid to remain is rather critical, both to avoid overflow and to provide sufficient depth of fluid to cover the light beam.

It might seem that, as between (2) and (3), (2) is the more satisfactory. Nevertheless, it was felt that in principle (3) was the right approach, and steps were therefore taken to remove the disadvantages mentioned above. The devices which evolved enable rapid and convenient scanning of extended wavelength ranges without the necessity of diluting the solutions, or even of removing them from the cuvette. The descriptions are of actual models which have been in use for more than a year. Possible modifications suggested by this experience are noted where appropriate.

(a) General Purpose Cell

This is the standard cuvette modified to permit the rapid insertion of a spacer without overflow, and is made by fusing a reservoir on to the end of the cuvette, as shown in Fig. 1. The exact dimensions of the reservoir may vary with the design of the spectrophotometer, and any optically suitable material may be employed for the cell as a whole, namely, 'Pyrex', silica, 'Perspex', etc. The prototype, intended for use with the Beckman *DK*-1 recording spectro-

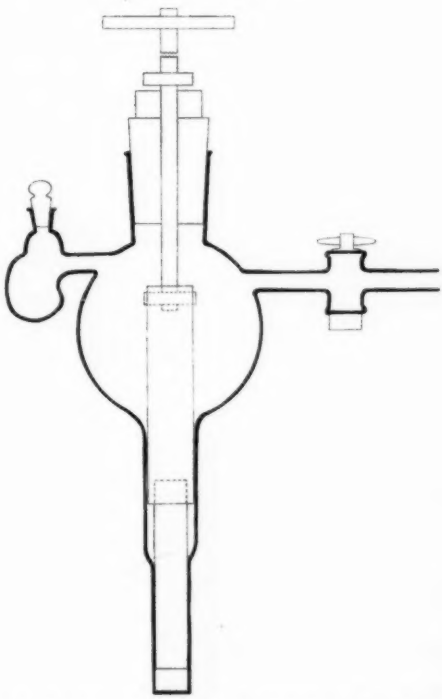

Fig. 2. Gas-tight cell. Optical path at right angles to plane of paper

photometer, was of 'Pyrex' glass and had a reservoir which was squared-off parallel to the sides of the cuvette. The reservoir would take up about 2 ml. of fluid. A later model of silica has a reservoir of greater cross-sectional area which will take up a full 4 ml. For these cells a silica spacer of the conventional type, that is, a block of silica 9 × 9 × 45 mm., was adapted by adding a cap of 'Teflon' to provide a handle for withdrawing the spacer though, again, any optically suitable material may be utilized. This was done by making a tight push-fit with the spacer into a piece of 'Teflon' tubing of the appropriate internal diameter. (It may be noted in passing that the complete unit is very similar in principle and in form to the cups used in colorimeters of the Klett type.)

(b) A Gas-tight Cell

The idea of attaching an optical cuvette to a gas reservoir, or tonometer, for observing reactions with gas mixtures goes back at least to Conant's work[1] in the late 1920's. Since then, the use of a Thunberg tube with a sealed-on cuvette has become standard, since this enables further reagents to be added during the course of the experiment if necessary. The most sophisticated designs of this type appear to be those of Keilin and Wang[2] and of Rossi-Fanelli and Antonini[3], which incorporate a mobile spacer. The spacer, however, is not readily controllable from the outside, particularly once the unit is *in situ* in the spectrophotometer. The device described here incorporates a spacer which may be raised or lowered at will from the outside. It consists (Fig. 2) of a standard 'Pyrex' cuvette to which is sealed a glass bulb of about 70 ml. internal volume. A side-arm bulb of about 2 ml. volume is provided for adding further reagents, and a two-way 'Teflon' tap controls filling and evacuation of the whole unit. A standard spacer of the type mentioned above was pushed into a length of 'Teflon' tubing suspended from a cross-piece at the bottom of a stainless steel shaft. The 'Teflon' was free to swing on the cross-piece and this provided the slight amount of play necessary to ensure that the spacer never became jammed in the cuvette through error in the alignment of the spacer assembly. The stainless steel shaft passed through an 'Asco' gland (A. F. Smith Co., Rochester, N.Y.) fitting into a standard ground glass joint at the top of the reservoir. The 'Asco' gland is a 'Teflon' stopper which forms a gas-tight seal, outside and inside, by means of rubber O-rings. The inner O-ring may be tightened against the shaft by means of an adjusting device. The pressure on this O-ring was so arranged that the shaft could be raised or lowered easily, and yet would not move from the set position even with an internal vacuum.

If only the vacuum from a water pump is employed, the 'Teflon' stopcock and the rubber O-rings will maintain the desired gas pressure for a considerable time. Hence, one can avoid the use of sealing grease, thereby eliminating possible contamination of the solution, and making for quicker and easier cleaning. In principle, one should be able to use the cell at much lower pressures, at least down to 0·1 mm. of mercury; but it does not seem possible to quote a lower limit at present owing to individual variations among 'Teflon' stopcocks.

With the complete cell one can, for example, determine the spectrum of oxyhæmoglobin from 350 to 700 mμ in one operation, followed immediately by the spectrum of deoxygenated hæmoglobin over the same range, produced by adding a little sodium dithionite from the side-arm. Both spectra can be

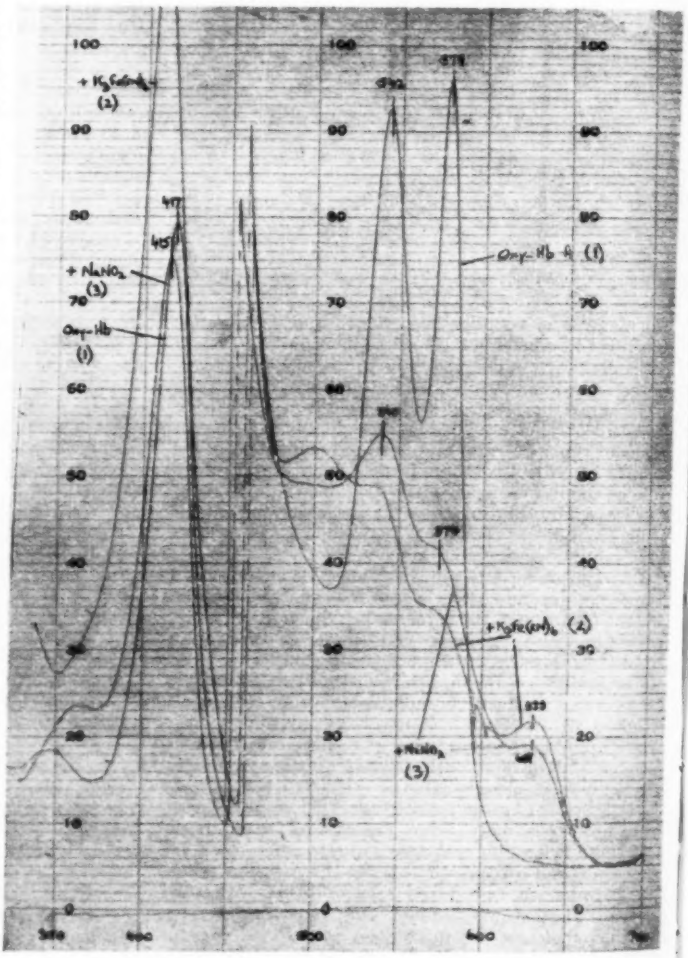

Fig. 3. Traces obtained using the general purpose cell in conjunction with a recording spectrophotometer. Spacer inserted at about 460 mμ in each case. Ordinates: optical density. Abscissæ: wave-length in mμ. (1) Human oxyhæmoglobin; (2) acid methæmoglobin from (1) + solid potassium ferricyanide; (3) nitrite methæmoglobin from (2) + solid sodium nitrite

taken under nitrogen, so reducing the liability of oxidation, and this can be replaced by carbon monoxide to give the carbon monoxide–hæmoglobin spectrum without disturbing the solution. The same sequence of operations would be considerably longer and more cumbersome to carry out by the usual procedures, and there would be much greater risk of artefacts due to the presence of oxygen and the mechanical agitation involved.

Practical Points

With both types of cuvette, the use of standard spacers, rather than spacers designed specifically for the purpose, meant that the rise and fall of the spacer had to be carefully checked to ensure that it would enter and leave the light beam completely. This was done by moving the spacer slowly into the light beam and observing the resultant optical density changes. The limits of the beam can be very accurately noted in this way, and were recorded for further work by marking the cuvettes themselves. Also as a result of using standard spacers, in both cells the spacer would no longer go to the bottom of the cuvette and a minimum volume of 2·5 ml. of solution was required. Ideally, one would use longer than normal spacers, which would obviate the need for an added 'handle', and would make possible the use of the cells with very small volumes, that is, a minimum of about 1 ml. of fluid if the cells were raised relative to the light beam.

Fig. 3 shows an actual unretouched trace which demonstrates the capabilities of this technique when used in conjunction with a recording spectrophotometer. Three successive spectra were recorded: oxyhæmoglobin; methæmoglobin formed by the addition of a little solid potassium ferricyanide; and nitrite methæmoglobin formed by the further addition of a little solid sodium nitrite. The traces were recorded using the standard cuvette in a Beckman *DK*-1 recording spectrophotometer. At the point where the optical density was rising sharply in the region of 460 mμ the cell compartment cover was quickly removed and the spacer inserted, the cover then being replaced. The very smooth transition, and the ease with which the complete spectrum may be visualized, should be especially noted. Similar results were obtained with the gas-tight cell, save that it was unnecessary to move the cell compartment cover. The exact procedure to be adopted when inserting the spacer will depend on the instrument being used, and its position in relation to outside light sources. With the *DK*-1, as used in this work, it was possible to have the compartment cover partly open the whole time without being able to detect stray light in the machine. In less-favourable situations, the cell could easily be handled through a black cloth, or placed under a light-tight box standing over the cell compartment.

Both cells have been in fairly continual use for about a year without showing any signs of scratching to either cuvettes or spacers. Neither has there been the slightest difficulty with bubbles adhering to the optical faces. In the orginal gas-tight cell, the 'Asco' gland used was a size 24/40 with 6-mm. bore and standard taper, the smallest then available. A 10/30 gland with standard taper and 6-mm. bore is now available, and this is a most convenient size. Apart from this, two immediate modifications, in principle rather than design, have been suggested in use. The primary one is that the useful range of the cells would be enormously extended in both the ultra-violet and infra-red regions if silica cuvettes were employed (in conjunction with silica spacers). There is no serious difficulty in doing this, and a standard general purpose cell has already been constructed in this material. Secondly, the usefulness of the cells could be further extended by the provision of spacers with 9 mm. optical path in one direction, and 8 mm. optical path at right angles. In the case of the hæmoproteins mentioned here, the 8-mm. spacing would enable one to examine the protein band around 280 mμ at a more suitable optical density in a continuous scan from 700 mμ down to 220 mμ.

The devices described here were developed and constructed during tenure of National Science Foundation grant *G* 7657 and U.S. Public Health Service grant *A* 3187 (*C*3); and it is a particular pleasure to acknowledge the interest and encouragement of Prof. Philip George.

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EVIDENCE FOR THE PHOTO-ASSOCIATION OF AROMATIC HYDROCARBONS IN FLUID MEDIA*

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THE pattern of singlet and triplet π-electron energy stages of an aromatic molecule A ($^1\Sigma$) shown in Fig. 1*b* closely follows that for an atom with ground state ^1S. A logical development of this pattern should lead us to expect the existence of an electronically excited dimeric state A_2^* in fluid media (Fig. 1*b*), corresponding to the stable excited diatomic molecule formed collisionally from excited and unexcited atoms of an inert gas[1], for example:

$$He^* + He \rightarrow He_2^* \rightarrow He + He + h\nu \text{ (continuum)}$$

(the asterisk denotes electronic excitation). Moreover, the radiative relaxation of the photo-associated species A_2^* to a dissociated ground state (process 4 of Fig. 1), like that of its diatomic counterpart, should be accompanied by a structureless emission at longer wave-lengths than the corresponding molecular fluorescence, whereas the reverse process of absorption will be limited by the extent to which association occurs in the ground state.

Such an emission was first reported by Förster and Kasper[2] as a broad, blue, structureless band, which replaces the violet, structured fluorescence of dissolved pyrene as its concentration is increased. No cryo-

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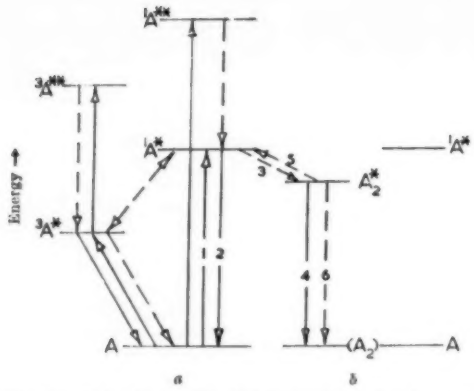

Fig. 1. Electronic energy-levels of aromatic hydrocarbon A in monomeric (*a*) and dimeric (*b*) forms. Radiative and non-radiative transitions are indicated by solid and dashed lines, respectively

scopic evidence was obtained for the association of unexcited molecules even in the most concentrated solutions and the molecular absorption spectrum remains unchanged over the range of concentrations investigated. Since the absorbing species is monomeric the operative processes may be represented by the scheme:

$$A + h\nu \xrightarrow{1} A^* + A \xrightarrow{3} A_2^* \xrightarrow{4} (A_2) + h\nu\,\text{(blue)}\ \text{(structureless)}$$
$$\downarrow 2$$
$$A + h\nu\,\text{(violet)}\ \text{(structured)}$$

where the asterisk denotes an electronically excited state and the processes are numbered according to Fig. 1. The rate constant[3] for process 4 is $\sim 10^6$ sec.$^{-1}$ although a small component of the blue emission with a lifetime of $\sim 10^{-3}$ sec. has been observed in degassed solutions[4]. 3,4-Benzpyrene[5] and a few other pyrene derivatives[3] show a similar effect to a smaller extent.

If the absence of dimer emission in other aromatic systems is due to the relatively high probability of competing processes, the evidence for photo-association must be based on these processes which include:

(*a*) dissociation (process 5) proposed[6] to account for delayed fluorescence observed in the vapours of a number of aromatic compounds recorded in Table 1.

$$A_2^* \rightarrow A^* + A \qquad (5)$$

In each case the spectrum of delayed fluorescence is identical with that of the molecular fluorescence originating from the lowest excited molecular singlet state A^*, but the lifetime is about 10^{-3} sec.; the ratio of delayed to total fluorescence intensities increases with the pressure of the vapour, and the small temperature dependence of the delayed component lifetime is incompatible with a mechanism in which the fluorescent state is thermally re-populated from the lowest triplet level. The absence of dimer emission under these conditions indicates that its radiative lifetime is at least 10^{-3} sec. and is probably at least ten times longer. A similar effect reported by Kautsky[9] in 1935 for very dilute solutions of a number of dyes, hæmatoporphyrin and chlorophyll, was interpreted in the same way.

Since process 5 requires an activation energy, it is less predominant at room temperatures than:

(*b*) internal conversion (process 6) which leads to:

$$A_2^* \rightarrow (A_2) \rightarrow A + A \qquad (6)$$

the overall self-quenching of fluorescence in solution[10] exhibited by several of the aromatic hydrocarbons listed in Table 1. In the vapour phase process 5 is dominant in the majority of cases investigated under the conditions of observation; however, a quantitative treatment[13] of the simultaneous self-quenching and delayed fluorescence observed in anthracene vapour has shown that processes 5 and 6 are mutually competitive, which suggests that photo-association is an essential step in the self-quenching mechanism. A value of 7·6 kcal./mole obtained[13] for E_5–E_6, the difference in activation energies of processes 5 and 6, provides a lower limit to the heat of photo-association in this case, and since the rate constant for process 6 is $\sim 10^9$ sec.$^{-1}$ at 220° C. (ref. 13), the excited dimeric state of anthracene should have a lifetime of at least 10^{-3} sec. with respect to internal conversion in solution at room temperature. The remarkable duration of this electronically excited species should promote:

(*c*) efficient energy transfer to an acceptor B (process 7):

$$A_2^* + B \rightarrow (A_2) + B^* \qquad (7)$$

which has been proposed[14] to account for the identity of the anthracene vapour fluorescence spectrum and the delayed fluorescence spectrum of phenanthrene vapour containing not more than 10^{-4} mm. (about 10^{-3} mole/l.) of anthracene. If the lifetime of phenanthrene in its photo-associated state is $\geqslant 10^{-3}$ sec., this concentration of anthracene acceptor is sufficient to account for the effect observed, which provides an upper limit of 5·4 kcal./mole for the heat of formation of the phenanthrene dimer. Furthermore, the efficiency of process 7 in this case is consistent with the conservation of overall spin angular momentum which is only possible if the photo-associated donor is in a singlet state. The pyrene-sensitized delayed fluorescence of naphthacene vapour present in trace quantities requires a similar interpretation[8] since delayed fluorescence has not been

Table 1. Evidence for Excimer Formation in Aromatic Hydrocarbons

Molecule	Emission (process 4)	Delayed fluorescence (process 5)	Self-quenching (process 6)	Sensitized delayed fluorescence (process 7)
Benzene	No (ref. 2)		Yes (ref. 10)	
Naphthalene			Yes (ref. 10)	
Anthracene	No (ref. 2)	Yes (refs. 7, 8)	Yes (refs. 10, 15)	
Phenanthrene		No. (ref. 14)		Yes (ref. 14)
Fluorene		Yes (ref. 7)		
Carbazole		Yes (ref. 7)		
Naphthacene		No (ref. 8)	Yes (ref. 11)	
Pyrene	Yes (ref. 2)	Yes (ref. 6)		Yes (ref. 8)
3,4-Benzpyrene	Yes (ref. 5)			
Perylene		Yes (ref. 6)	Yes (ref. 15)	
1,12-Benzperylene		Yes (ref. 8)		
Coronene			Yes (refs. 12,15)	
Anthanthrene		Yes (ref. 8)		
9,10-Diphenylanthracene	No (ref. 8)	No (ref. 8)	No (refs. 13, 15)	

observed from naphthacene vapour alone where self-quenching is extremely efficient[11]. In this case the difference in energies of pyrene and naphthacene molecules in their lowest excited singlet states provides an upper limit of 15·2 kcal./mole for the heat of photo-association of pyrene.

If these competing processes are acceptable criteria for the formation of an excited dimeric species by the collisional process 3, the failure of 9,10-diphenylanthracene to exhibit either delayed fluorescence in the vapour phase[8] or self-quenching in solution[15] suggests that the photo-association of this non-planar molecule is sterically hindered. However, the observations listed in Table 1 indicate that the photo-association of planar aromatic molecules is of general occurrence, and, despite the absence of direct evidence in the majority of cases, it must be concluded that in fluid media the stable form of planar aromatic molecules in their excited singlet states is dimeric. The term 'excimer' has been proposed[4] to distinguish this state from the excited dimer of certain dyes which is produced by absorption from a stable dimeric ground state[16] and which has the quite different properties[4] shown in Table 2. Excimer formation, like triplet state population, is not necessarily limited to aromatic systems.

Table 2. PROPERTIES OF EXCIMER AND EXCITED DIMER

	Excimer	Excited dimer
Ground state Dimerization	dissociated follows act of light absorption	stable at room temperature precedes act of light absorption
Radiative relaxation	forbidden	allowed*
Corresponding absorption	not observed	increases with concentration

* According to intensity of corresponding absorption (ref. 16).

Although photo-association appears to be of general occurrence unless sterically hindered, the intermolecular binding forces will depend on the electronic properties of the molecules involved and may be of less general origin. A resonance splitting of the two-fold degenerate electronic levels of identical molecules in a parallel sandwich configuration has been treated by Hoijtink[17], who concludes that the stable state is a singlet with an energy some tenths of an electron-volt below that of the corresponding state of the monomer, and that owing to its centre of symmetry the radiative relaxation to a dissociated ground state is orbitally forbidden; these conclusions are in qualitative agreement with the experimental observations although the symmetry restriction requires a strictly parallel configuration of the aromatic planes which is unlikely to persist for 1 msec. in the vapour phase or even in solution at room temperature.

An explanation of excimer emission in solution with $k_4 \sim 10^6$ sec.$^{-1}$ and delayed fluorescence in the vapour where $k_5 \sim 10^3$ sec.$^{-1}$ appears to require at least two states of the pyrene excimer between which interaction is weak or dependent on temperature: Murrell has shown (in a private communication) that the charge-transfer state A^+A^- of pyrene, corresponding to an interplanar distance of $\leqslant 3{\cdot}5$ Å., lies below the first excited singlet state of the monomer, in which case configuration interaction with the resonance state may be responsible for the exceptional radiative properties of this excimer in solution. A third possibility is the doubly excited state suggested by Hoijtink (private communication) to explain the remarkable resistance to radiative relaxation exhibited by excimers in the vapour state; Colpa[18] has pointed out that a doubly excited dimeric singlet state correlates with two molecular triplet states, and since the lowest excited singlet energy of molecules such as anthracene and naphthacene is almost exactly twice that of the corresponding triplet-level, the doubly excited state could be populated by a diabatic process following the collision of molecules in the ground and excited singlet states.

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STRUCTURE AND PROPERTIES OF THE FLAVOHÆMOPROTEIN, CYTOCHROME b_2 (L(+)-LACTATE DEHYDROGENASE OF BAKER'S YEAST) AND OF HÆMOPROTEIN, FLAVOPROTEIN AND APOPROTEIN DERIVATIVES

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CYTOCHROME b_2 was first observed in 1942 by Bach, Dixon and Keilin[1], and was partly purified by Bach, Dixon and Zerfas[2,3], who found that the cytochrome was closely associated with the lactate dehydrogenase of aerobic baker's yeast. In 1954 Appleby and Morton[4,5] reported the purification and crystallization of cytochrome b_2 and showed that it was identical with yeast L(+)-lactate-cytochrome *c* reductase (L(+)-lactate dehydrogenase). The crystalline enzyme was found to be a flavo-

Table 1. Nomenclature of Derivatives of Cytochrome b_2*

Name	Non-protein groups	L(+)-Lactate-cytochrome *c* reductase activity	Principal absorption bands (mμ)	Other properties
Type I cytochrome b_2	DNA FMN † Protohæm	Active	α, 557; β, 528; γ, 423·5; u.v., 265	Crystalline; molecular weight, 172,000
Type II cytochrome b_2	FMN Protohæm	Active	α, 557; β, 528; γ, 423·5; u.v., 269·5	Crystalline
Type III hæmoprotein-550	DNA Ferro-porphyrin *c*	Inactive	α, 550; β, 521; γ, 415; u.v., 257	Crystalline; reduced by cytochrome *c* reductases; oxidized by cytochrome *c* oxidase
Type IV hæmoprotein-557	Protohæm	Inactive	α, 557; β, 528; γ, 423·5; u.v., 276	Poorly crystalline; slightly autoxidizable
Type V flavoprotein	DNA (?) FMN	Inactive	Oxidized, 445; 375; u.v., 266	
Type VI apoprotein	DNA	Inactive	u.v., 260 mμ	

* The full name for protein derivatives should include reference to the parent material—thus 'type III cytochrome b_2–hæmoprotein-550'.
† Riboflavin phosphate.

hæmoprotein since it contained equimolecular amounts of riboflavin phosphate and of protohæm as prosthetic groups[4,6,7]. In addition, the enzyme contained a constant proportion of a deoxyribonucleic acid (DNA)[8,9] of small molecular weight as estimated by end-group analysis[9].

The DNA-containing cytochrome b_2 is regarded as the naturally occurring form of the enzyme, and is now known as type I-cytochrome b_2 (ref. 10) (Table 1). The enzymic[4,5,11], chemical[4,7–9,11,12] and physico-chemical[4,8,11,13,14] properties of crystalline type I cytochrome b_2 have been described. The enzyme is homogeneous with respect to sedimentation as indicated by boundary analysis[15] of sedimentation patterns of the enzyme in lactate-pyrophosphate-ethylenediamine tetraacetate buffers, and by sedimentation patterns after treatment with 2·6 *M* and 5 *M* urea[13–15]. Only a single protein component has been detected by moving-boundary electrophoresis[4,8,14]. Although the possible existence of a flavoprotein and a separate hæmoprotein in crystalline type I cytochrome b_2 was recognized by Appleby and Morton[4], continued work since 1954 has produced no evidence for this hypothesis. Boeri *et al.*[16] used a non-crystalline preparation and confirmed the finding of Appleby and Morton[4] that cytochrome b_2 is a flavohæmoprotein identical with yeast L(+)-lactate-cytochrome *c* reductase.

Further information concerning the structure and properties of cytochrome b_2 has been obtained by modification of type I cytochrome b_2. A series of derivative proteins have been prepared, some in the crystalline state. Table 1 gives the nomenclature adopted for the various modified proteins so obtained. Both the hæmoprotein and flavoprotein derivatives are inactive as lactate-cytochrome *c* reductases, as is the apoprotein (the protein derivative free of both prosthetic groups). Lactate-cytochrome *c* reductase activity has been re-established by the re-combination of riboflavin phosphate with the hæmoprotein derivative and by the stepwise addition of protohæm and of riboflavin phosphate to the apoprotein; either alone is ineffective. The two prosthetic groups are attached at the active centre of the one protein; the presence of both groups is essential for enzymic reduction of cytochrome *c*. It is concluded that reduction of cytochrome *c* by the native enzyme involves intra-molecular electron transfer from the flavin to the hæm prosthetic group.

For these investigations, twice-crystallized type I cytochrome b_2 was the usual starting material. Unless otherwise specified, all solutions contained 0·1 m*M* ethylenediamine tetraacetate, and all work was carried out under anaerobic conditions at 0–2° C., with de-aerated solutions equilibrated with oxygen-free nitrogen. Solutions were usually prepared by dissolving crystals of type I cytochrome b_2 in 0·5 *M* sodium lactate–0·04 *M* sodium pyrophosphate–0·1 m*M* ethylenediamine tetraacetate, *p*H 6·8; the protein concentration was 15–25 mgm./ml. Enzymic activities were estimated at 25° C. and as described by Appleby and Morton[5], and by Morton and Shepley[10], rates being calculated from changes in optical density due to the enzyme during the first minute. Protein concentration was calculated from the optical density of the reduced form of the pigment[5] or by the method of Lowry *et al.*[17]. Fluorescence was observed visually on irradiation with a 'Hanovia' lamp at about 360 mμ, or measured with an EEL fluorimeter.

Crystalline forms of type I cytochrome b_2. The ferrocytochrome is regularly obtained as crystals which appear as shallow bipyramids belonging to the tetragonal system[4,5]. Crystals have also been obtained by precipitation with acetone; according to the conditions, each of the crystalline forms shown in Fig. 1 may occur. The crystals shown in Fig. 1*B* appear to be macles (hemitropes), formed by co-crystallization of the protein and salt developing on a common crystal face. The ferricytochrome has also been obtained as tan-coloured bipyramids, in contrast to the pink-coloured crystals of the ferrocytochrome. The crystals of the oxidized enzyme give solutions which are burgundy red when concentrated and brown when dilute; the absorption spectrum of a concentrated solution shows a band rising from about 620 mμ to a maximum at 533 mμ, with a minimum at 510 mμ, and a distinctive shoulder at 563 mμ. The peak at 532–535 mμ and the shoulder at 563 mμ are seen as absorption bands in a micro-spectroscope.

The distinctive crystalline forms of type I cytochrome b_2 depend on the presence of the DNA, flavin and hæm group in the protein; dissociation of any one of these will either prevent crystallization under the conditions used or change the crystalline form obtained. By suitable treatments which destroy enzymic activity, but which leave the prosthetic groups intact on the protein, enzymically inactive crystals of cytochrome b_2 have been obtained.

Crystalline type II cytochrome b_2. The deoxyribonucleic acid of native cytochrome b_2 has a marked influence on the electrophoretic mobility, the sedimentation coefficient, and the dependence on concentration of the sedimentation coefficient of the enzyme[14,15]. However, removal of the deoxyribonucleic acid by salt-precipitation of the protein does not interfere with the dehydrogenase activities of the protein[5]. Crystallization of the resultant type II cytochrome b_2 as large bi-pyramidal prisms belonging to the hexagonal system has been described by Morton and Shepley[10]. Type II cytochrome b_2 has equimolecular

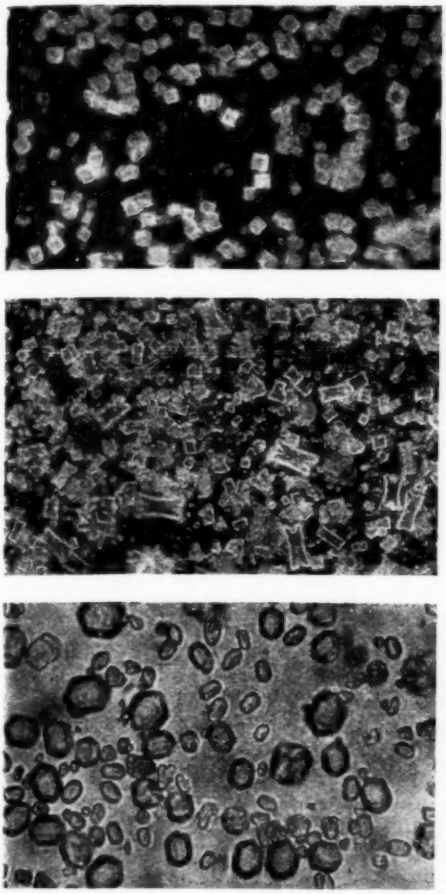

Fig. 1. Different crystalline forms of type I cytochrome b_2 obtained by acetone precipitation; the forms vary according to the conditions used for preparation. *A*, × 200; *B*, × 200; *C*, × 300

proportions of riboflavin phosphate and of protohæm and similar specific activities to type I cytochrome b_2 (refs. 10 and 11).

The occurrence of cytochrome b_2 as a crystalline protein, which may be obtained in a number of characteristic crystalline forms, each having equimolecular proportions of protohæm and of riboflavin phosphate, strongly supports the extensive physico-chemical evidence for the homogeneity of the protein; fortuitous association of a flavoprotein enzyme and of a separate hæmoprotein in cytochrome b_2 obtained by so many different purification and crystallization procedures becomes so improbable that this hypothesis may now be dismissed.

Cytochrome b_2 *- hæmoprotein - 550 (type III hæmoprotein).* This is an enzymically inactive, flavin-free derivative of the enzyme. It was obtained from recrystallized type I cytochrome b_2 fortuitously, by acetone precipitation under conditions to be described elsewhere[18], as somewhat irregular crystalline plates.

The absorption spectrum of both the reduced and oxidized forms are remarkably similar to those of horse-heart and of yeast cytochrome *c*, but the spectra differ considerably in the ultra-violet region, largely due to the DNA-component of type III hæmoprotein. The extinction coefficients, expressed per mole of protein-bound hæm, are substantially the same for the α-, β- and γ-bands of the enzymically reduced cytochrome *c* and the type III hæmoprotein. The type III hæmoprotein derivative resembles cytochrome *c* in the following chemical properties: the pyridine hæmochromes are qualitatively identical for the α-, β- and γ-bands; the prosthetic group is not split by acid-acetone or acid-methylethylketone; treatment with silver sulphate in acetic acid causes distinctive changes in spectra, consistent with cleavage of thio-ether bonds, although the reaction products have not yet been identified. Like cytochrome *c* it is not autoxidizable at *p*H 7 and does not combine with carbon monoxide; it is reduced by ascorbic acid and by cytochrome b_2 (lactate-cytochrome *c* reductase); the reduced hæmoprotein is oxidized by cytochrome *c* oxidase from heart-muscle. However, whereas horse-heart cytochrome *c* has an isoelectric point of 10·2, type III hæmoprotein-550 has similar electrophoretic mobility to type I cytochrome b_2. It migrated very slowly in 12 hr. on electrophoresis at *p*H 6·8 in 0·3 *M* sodium lactate–0·05 *M* sodium pyrophosphate–0·1 m*M* ethylenediamine tetraacetate, *p*H 6·8, ionic strength 9·63, as a single component. The sedimentation coefficient was 3·4 Svedbergs (at 1·3 mgm. protein/ml.), and thus approximately half that of type I cytochrome b_2.

In view of the remarkable similarity to cytochrome *c*, including reduction by cytochrome *c*-linked dehydrogenases, and the available chemical evidence, it is tentatively concluded that cytochrome b_2-hæmoprotein-550 is a derivative of type I cytochrome b_2 containing at least one thio-ether bond from the protein to the hæm prosthetic group. It is likely that (*a*) reactive sulphydryl groups of cytochrome b_2 were exposed, possibly arising from cleavage of disulphide bonds in the original material; (*b*) these sulphydryl groups attacked the vinyl side-chains of the protohæm prosthetic group during acetone treatment of the protein, forming a thio-ether bond to the protein. This work will be described elsewhere[18], since it provides a possible model for physiological formation of cytochrome *c* from a protohæmoprotein precursor. The formation of type III hæmoprotein gives further support for the importance of highly reactive sulphydryl groups in cytochrome b_2[7,13,14]. Moreover, the similarity of the absorption spectra of cytochrome *c* and of type III hæmoprotein in the visible and near ultra-violet regions shows that similar amino-acid residues are co-ordinated with the iron atoms of the hæm residues in cytochrome *c* and in cytochrome b_2. Whereas cytochrome b_2 has two flavin and two hæm groups per molecule, and a molecular weight of 172,000 (refs. 14 and 19) hæmoprotein 550 appears to have one hæm group per molecule, and probably has a much lower molecular weight.

Cytochrome b_2 *- hæmoprotein - 557 (type IV hæmoprotein).* This modification of type I cytochrome b_2 was obtained by removal of the flavin group of the enzyme by ammonium sulphate precipitation at *p*H 3·2. Loss of enzymic activity was proportional to the amount of riboflavin phosphate removed in the supernatant. The final product had about 0·02 times the amount of flavin in type I cytochrome b_2, and was correspondingly inactive with cytochrome *c*, ferricyanide, and dyes as acceptors for the dehydro-

genase. The ferro-hæmoprotein has absorption maxima at 557, 528, 423·5 and 350 mμ; it does not combine significantly with carbon monoxide; and is only slightly autoxidizable, indicating a minimum of denaturation during the preparation. Rather poor crystals, which appeared as rectangular plates, were obtained by slow precipitation of this hæmoprotein with acetone. The sedimentation coefficient (1·3 mgm./ml.) was 7·94 Svedbergs; only a single protein component was detected on sedimentation. The prosthetic group is protohæm, as in cytochrome b_2. As shown here, enzymic activity of the enzymically inactive hæmoprotein is partly restored by recombination with riboflavin phosphate.

Cytochrome b_2-hæmoprotein-557 is probably closely related to a hæmoprotein which was crystallized by Okunuki and co-workers in 1957 [10]. The latter material, however, was highly autoxidizable; the effect of carbon monoxide was not reported. Although widely quoted as evidence for the existence of separate flavoprotein and hæmoprotein ('cytochrome b_2') moieties in L(+)-lactate-cytochrome *c* reductase of yeast, it is clear that the material obtained by Yamashita *et al.* was a derivative of native cytochrome b_2 obtained by denaturation of the enzyme during isolation[7,11].

Cytochrome b_2 *- flavoprotein (type V flavoprotein).* The lability of the flavin group of cytochrome b_2 creates considerable difficulty in selective removal of the hæm group without simultaneous depletion of riboflavin phosphate. However, it was found that the hæm group was quantitatively removed without extraction of the riboflavin phosphate by the following procedure. About 5 mgm. of freshly prepared crystals of type I cytochrome b_2 were dissolved in approx. 3 ml. of 0·1 *M* potassium cyanide at 0° C., and acetone pre-cooled to − 20° C. was slowly added to the solution at − 2° to − 5° C. until all the protein was precipitated. The yellow precipitate was washed with 60 per cent (v/v) acetone at − 15° C. and dissolved in about 2 ml. of 0·1 *M* sodium lactate, *p*H 7·5. Some insoluble protein was removed by centrifuging. The solution was dialysed against 0·1 *M* sodium lactate – 0·05 *M* sodium pyrophosphate – 0·1 m*M* ethylenediamine tetraacetate for 4 hr. Some insoluble protein was removed by centrifuging. Application of this procedure for removal of the prosthetic group of other hæmoproteins is being examined.

The flavoprotein derivative so obtained has 1 mole of riboflavin phosphate per 80,000 gm. as does native cytochrome b_2; however, the flavin group of the derivative, unlike that of native cytochrome b_2, is fully fluorescent. The absorption spectrum of the type V-flavoprotein shows maxima at 266, 375, and 445 mμ; there is a slight shoulder at 418 mμ, probably due to contaminant hæm material. These maxima correspond with those observed in the difference spectrum between native cytochrome b_2 and a preparation treated so that the hæm group was reduced but the flavin oxidized; a similar difference spectrum was found by Hasegawa and Ogura[31].

The type V flavoprotein was enzymically inactive with lactate as substrate and with cytochrome *c*, ferricyanide, or 2:6-dichlorophenol indophenol as acceptors; enzymic activity was not restored by incubation at 0° C. with protohæmin. The material is readily denatured with simultaneous dissociation of the flavin group.

Cytochrome b_2 *- apoprotein (type VI - apoprotein).* Simultaneous removal of both the flavin and hæm prosthetic groups was accomplished by treatment of type I cytochrome b_2 crystals with acid-acetone at − 40 to − 60° C. The colourless apoprotein has a single absorption peak at about 260 mμ. Although there was considerable amount of denaturation, some of the protein was readily soluble in dilute lactate-pyrophosphate buffer. With this material, recombination of flavin and hæm groups partly re-established enzymic activity, but a fraction of the protein only soluble in more concentrated buffer was completely inactive.

Restoration of lactate dehydrogenase activity in hæmoprotein-557 and in apoprotein from cytochrome b_2. Table 2 shows that the inactive, type IV hæmoprotein-557 may be re-activated by incubating with riboflavin phosphate. The resultant protein catalyses reduction of cytochrome *c*, ferricyanide, 2 : 6-dichlorophenol indophenol and phenazine methosulphate. Whereas about 12 per cent of the ferricyanide reductase activity of type I cytochrome b_2 was recovered, addition of the flavin group restored only about 1 per cent of the cytochrome *c* reductase activity. Thus, as compared with reduction of ferricyanide, reduction of cytochrome *c* involves a more specific orientation of the two prosthetic groups.

Table 2. Restoration of Lactate Dehydrogenase Activity of Type IV Hæmoprotein-557

Acceptor	Hæmoprotein-557	Hæmoprotein-557 plus riboflavin phosphate
Cytochrome *c*	20	120
Ferricyanide	< 10	1,640
2 : 6-Dichlorophenol indophenol	7	90
2 : 6-Dichlorophenol indophenol plus phenazine methosulphate	10	140

The hæmoprotein solution was incubated with riboflavin phosphate (approx. 3 moles per 80,000 gm. of protein) at *p*H 7, and at 0° C., for about 2 hr. Activities were estimated as described by Morton and Shepley (ref. 10); they are given as μmoles of acceptor reduced per hr. per ml. of solution of hæmoprotein.

Further information concerning the functions of the two prosthetic groups was obtained from attempts to re-establish activity with a type VI apoprotein. The inactive material was incubated at *p*H 7·5 with either 1·5 moles of protohæmin per 80,000 gm. of protein or with 3 moles of riboflavin phosphate per 80,000 gm. of protein, or successively with each of these for 6–8 hr. at 0° C. At the end of this period (about 16 hr.), activities of the various materials, alone and in several combinations, were tested.

The apoprotein-hæmin and apoprotein-flavin incubates were inactive. Moreover, there was no reduction of any acceptor in the presence of lactate when portions of both the apoprotein-hæmin and the apoprotein-flavin preparations were added to the reaction mixtures. However, the preparation which had been incubated with hæmin for 6 hr. and afterwards with riboflavin phosphate showed a considerable activity with each acceptor. Fig. 2 shows the restoration of activity with cytochrome *c* as acceptor; similar results were obtained with ferricyanide and 2 : 6-dichlorophenol indophenol. Significantly, no activity was found in the preparation in which the addition of hæmin followed the addition of riboflavin phosphate. In a separate experiment, it was shown that some activity was recovered after incubation with both hæmin and riboflavin phosphate at the one time.

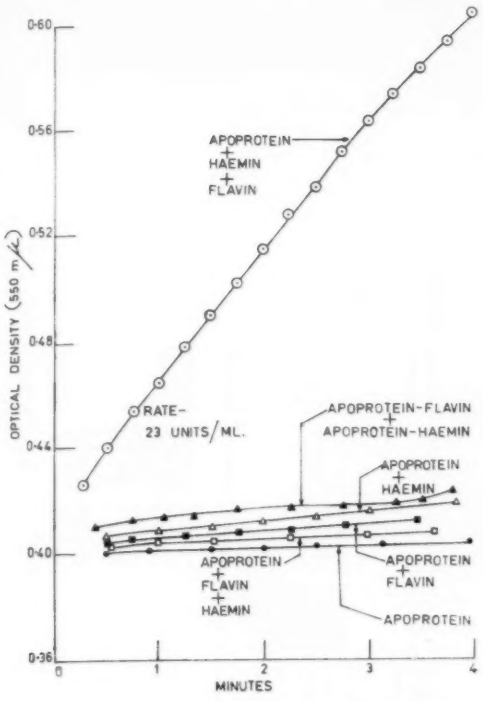

Fig. 2. Restoration of lactate-cytochrome *c* reductase activity of type VI apoprotein by successive incubation with protohæm and riboflavin phosphate. Activity tests were carried out as described by Morton and Shepley (ref. 10); approximately 5 μgm. of protein were used in each test

The recombination of the apoprotein and hæmin forms a hæmoprotein with an absorption spectrum which closely resembles that of type IV hæmoprotein-557. The re-activated protein formed by the further addition of riboflavin phosphate differs from the native type I cytochrome b_2 in that it is strongly fluorescent, and only a portion of the added hæmin is reduced by lactate. This is consistent with the finding of about 1 per cent of the cytochrome *c* reductase activity of cytochrome b_2 in the recombined material. In view of the established lability of cytochrome b_2[3,7], and the rather drastic conditions needed for the removal of the hæm group from the enzyme, it is unlikely that recovery of activity can be further increased. Details of these and related investigations will be reported elsewhere[22].

Selective inhibition of lactate-cytochrome c *reductase activity of cytochrome* b_2. Of a number of compounds recently tested by Hallett and Morton[23], most (such as arsenite and *p*-chloromercuribenzoate) caused similar inhibition of enzymic reduction of ferricyanide and of cytochrome *c* catalysed by type I cytochrome b_2. However, amytal at about 2·6 m*M* concentration caused a substantial inhibition of reduction of cytochrome *c* but had no similar effect on reduction of ferricyanide. Although the possibility of combination of amytal with cytochrome *c* has not been eliminated, this seems most unlikely. This selective inhibition supports kinetic studies of Armstrong and Morton[14,15] showing that the reduction of ferricyanide and of cytochrome *c* involves different mechanisms.

Discussion

Warburg and Christian[24] in 1932 isolated the first flavoprotein enzyme, and these workers, and Theorell[25], showed that the flavin group could be removed under acid conditions with loss of enzymic activity, which was recovered by recombination of flavin with the apoprotein.

The acid-acetone treatment developed by Hill and Holden[26] for preparation of globin from hæmoglobin was used by Theorell[27] in 1940 to remove the hæm prosthetic group of peroxidase. Recombination of apoprotein and hæmin was accomplished after incubation at room temperature for some hours, with recovery of enzymic activity[27].

In the case of cytochrome b_2, an enzyme discovered in 1942 by Dixon[1-3], activity has now been shown to depend on the presence of both flavin and hæm prosthetic groups. Since apoprotein-hæmin material added with apoprotein-flavin material is inactive, arguments for a flavoprotein-hæmoprotein pair in cytochrome b_2 (ref. 4) can be dismissed.

Kinetic[11], inhibitor[23], and reactivation[22] investigations are consistent and confirm that reduction of ferricyanide (an artificial acceptor) and of cytochrome *c* proceed through different pathways, as proposed in 1955[6]. However, enzymic activity with any acceptor depends on the presence of the two prosthetic groups, which so clearly interact, and not on either group alone.

Cytochrome b_2 of yeast mitochondria is a distinctive type of dehydrogenase, having two molecules of riboflavin phosphate and two molecules of protohæm per enzyme molecule; together, riboflavin phosphate and protohæm constitute the essential prosthetic group of this enzyme.

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RESOLUTION OF PATHOLOGICAL HUMAN GAMMA-GLOBULINS BY STARCH-GEL ELECTROPHORESIS

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THE heterogeneity of the 7S normal human γ-globulins has been demonstrated by several techniques, including electrophoresis, convection, chromatography and biological assays[1–7]. On starch-gel electrophoresis employing the continuous buffer system of Smithies[8], the γ-globulins are known to migrate towards the cathode as a broad zone. In the discontinuous buffer system described by Poulik[9] most of these globulins move in the opposite direction. The γ-globulins of pathological human sera, analysed by the 'continuous' gel electrophoresis technique, have been shown to migrate essentially as a single zone located in different positions within the area of the normal γ-globulins[10,11].

We report here the resolution by starch-gel electrophoresis of the γ-globulins of certain pathological sera into several distinct bands.

The principal results of this work are presented in Fig. 1. The distribution of the γ-globulins of the five representative pathological sera differed significantly from one other. In the continuous borate buffer system these globulins of four of the five specimens migrated towards the cathode like the normal γ-globulins. The first pattern (Fig. 1*a*) shows the starch-gel electrophoretic analysis of the serum from a patient with cancer of the ovary and widespread metastasis. The remarkable finding is that the γ-globulins separated into seven bands. The next pattern (Fig. 1*b*) represents the gel electrophoresis of the serum of a patient with probable chronic myeloid leukæmia. The γ-globulins resolved into three zones of which the middle one appeared to be further separated into four sub-bands. The pattern of the serum of a patient with hypergamma-globulinæmia of four years duration but without clinically detectable disease is shown in Fig. 1*c*. The γ-globulin zone appeared very dense compared with that of normal serum (Fig. 1*N*) although their areas were about equal. The γ-globulins of another patient with unknown diagnosis displayed a similar pattern with three narrow heavy bands superimposed. The γ-globulins of a further patient (Fig. 1*d*) moved as a single rather narrow band to the cathode side of the normal γ-globulin area. Comparable findings have been reported earlier[10,11]. The fifth specimen was obtained from a patient with chronic normochromic normocytic anæmia of sixteen years duration. It was interesting to observe that the γ-globulins migrated towards the anode, that is, in the direction opposite to that of the movement of the normal γ-globulins, and exhibited a pattern (Fig. 1*e*) characterized by a very intensely coloured band in the slow α_2 ($S\alpha_2$) region. (It is worth mentioning that this patient is not unusually prone to infections and, therefore, must still have the benefit of antibody protection.) In addition, there were three minor zones distributed almost equally between the slot of application and the major γ-globulin band mentioned.

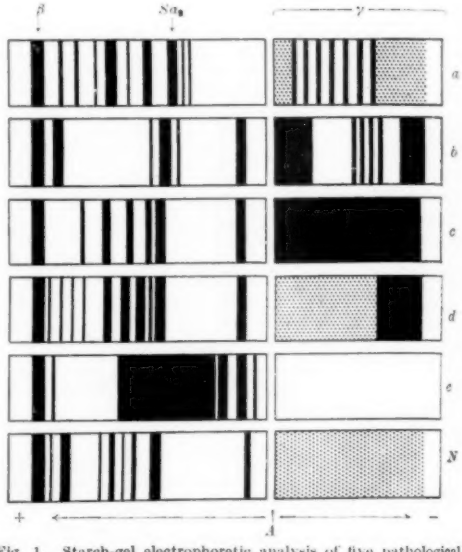

Fig. 1. Starch-gel electrophoretic analysis of five pathological sera (*a–e*). For comparison the corresponding pattern of normal human serum (*N*) is included, and the positions of the γ- (γ) and slow α_2- ($S\alpha_2$) globulins and transferrin (β) are given. The two arrows indicate the directions of movement of the proteins. '*A*' points to the slots of application. The starch-gel electrophoresis was performed in the cold room at 3° in *p*H 8·6 borate buffer at 20 m.amp. for 17 hr. The part of the patterns that shows the transferrin–pre-albumin region is not reproduced

Use of the discontinuous system of buffers led to the following findings: The γ-globulins of serum 1*a* entered the anodic side of the application slot and again separated into four bands which were located in the $S\alpha_2$ region. It appeared that additional γ-globulin bands were present in the haptoglobin area. Only traces of protein moved towards the cathode. Under the same conditions normal γ-globulin (Cohn's Fraction II) formed a broad zone in the $S\alpha_2$-haptoglobin area and further bands in the post-albumin region[12]. The γ-globulins of serum 1*c* displayed a pattern which was very similar to that observed in the continuous buffer system, except that a relatively smaller amount of protein seemed to be present, probably because the normal γ-globulins of this serum moved in the opposite direction. The γ-globulins of patient 1*d* migrated as in borate buffer towards the cathode, but a separation took place which led to one minor band preceding and two following the main zone. Moreover, additional lines were observed in the haptoglobin region. The γ-globulins of patient 1*e* displayed a pattern which was essentially the same as the protein distribution observed in the continuous system. Thus, judging from the behaviour of the γ-globulins of this limited series of sera, and as pointed out earlier by Poulik[13], the discontinuous buffer system leads to a higher resolu-

* Established investigator of the Helen Hay Whitney Foundation, New York.

tion than that resulting from the continuous system. Further, all γ-globulin bands obtained by the discontinuous system were located closer to the albumin zone as noted previously[13]. Nevertheless, certain pathological γ-globulins (1*b*, *c*, *d*) still migrated towards the cathode.

Paper electrophoresis at *p*H 8·6 in citrate barbiturate buffer of the sera investigated demonstrated in each case a significant increase of the proteins which migrated with mobilities corresponding to those of the γ-globulins. The increase over the normal value was 23–34 relative per cent. As judged by ultracentrifugal analysis the 7S-globulins of the same sera varied between 30 and 40 per cent. The 19S-component appeared approximately normal in concentration. Abnormal plasma proteins with sedimentation coefficients of 10 and 14S, respectively, were observed in one case (1*e*) only.

The present starch-gel electrophoretic investigation on pathological γ-globulins demonstrates that this procedure offers an advantage over paper electrophoresis in that certain abnormal γ-globulins can be resolved into several bands. This resolution seems to indicate that these pathological γ-globulins display a discontinuous spectrum with respect to apparent mobility in contrast to the continuous spectrum of the normal γ-globulins. If the mobility of the 7S-γ-globulins may be related to the net charge of these molecules, then this observation can be interpreted to mean that certain pathological γ-globulins are synthesized in such a way that they carry relatively unlike electrostatic net charges.

This work was supported by grants from the Lilly Research Laboratories, Eli Lilly and Co., and from the National Institutes of Health [*A*–3456(*C*1)], United States Public Health Service.

Note added in proof. After this communication had been submitted, an article by Owen, Got and Silberman (*Clin. Chim. Acta*, **3**, 605; 1958) came to our attention, in which the heterogeneity of the γ-globulins of patients with myeloma is described.

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GAMMA-GLOBULIN AND ACQUIRED IMMUNITY TO HUMAN MALARIA

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MALARIAL infection is followed by the development of immunity which is specific for the homologous parasite, but ineffective against heterologous strains and species[1–4]. The epidemiological effects of acquired immunity in man are seen among the inhabitants of hyperendemic malarious regions who show a consistent pattern of susceptibility to infection. Infants born in such regions are relatively resistant during the first three months of life and thereafter all children suffer severe and recurrent attacks of the disease. Clinical malaria becomes comparatively infrequent in later childhood and among adults is rarely seen in acute form[5,6].

The response of phagocytic cells in malarial infection was shown in early histological work[7], and for many years resistance to the disease was considered to be exclusively cellular in nature. In 1937, Coggeshall and Kumm[8] demonstrated the passive transfer of malarial immunity in rhesus monkeys and so established the presence of protective antibodies. However, the therapeutic effect of large doses of immune serum was evident only against minimal numbers of parasites and has not been demonstrated convincingly in man. The belief is therefore still prevalent that circulating antibodies play a relatively unimportant part in acquired malarial immunity[9].

Passive Transfer of Human Malarial Immunity

In this investigation the role of circulating antibody in malarial immunity has been studied in subjects living in The Gambia which is a hyperendemic area of West Africa. The therapeutic effect of γ-globulin prepared from the serum of apparently immune adults has been tested in young African children suffering from severe clinical malaria with dense parasitæmia. The γ-globulin prepared by chromatography on columns of diethylaminoethyl cellulose[10] was homogeneous on electrophoresis and in the ultracentrifuge appeared as a main peak with a sedimentation coefficient ($S^0_{20,W}$) = 6·78; an additional heavier component (10*S*) comprised less than 5 per cent of the total protein. The remainder of the protein eluted from the columns (referred to here as γ-free serum) was shown by serological tests to contain about 70 mgm. 7S γ-globulin and 20 mgm. 19S γ-globulin/gm. total protein.

Twelve children aged 4 months–2½ years (weight 5·4–12·6 kgm.) admitted with a clinical diagnosis of malignant tertian malaria and an initial parasitæmia of 10,000–230,000/c.mm. received γ-globulin by intramuscular injection at intervals of 8–24 hr. for 3 days. The total dose was 1·2–2·5 gm./child which

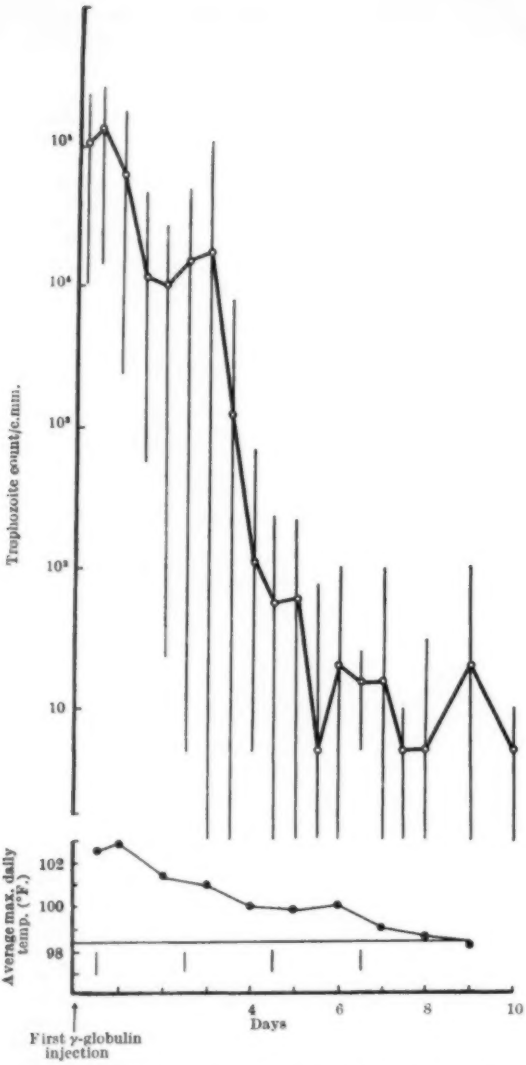

Fig. 1. Trophozoite counts (mean and range shown on a logarithmic scale) and average maximum daily temperatures in 12 children with acute *Plasmodium falciparum* malaria treated with immune γ-globulin. The time-scale refers to days after the first γ-globulin injection. Vertical lines below indicate approximate times of expected schizogony

was equivalent to 10–20 per cent of the recipient's own γ-globulin.

The infections were predominantly synchronous and γ-globulin therapy was usually started when the peripheral blood contained a high density of young ring forms. Blood examinations were made at 12-hourly intervals and the course of parasitæmia is shown in Fig. 1. By the fourth day after the inception of treatment parasite counts were always less than 1 per cent of the initial values; by the ninth day trophozoites were not detectable in 8 out of 12 cases and the maximum count was 80/c.mm. (Fig. 1). The fall in parasitæmia was accompanied by progressive alleviation of clinical illness although temperatures did not usually return to normal before the seventh day (Fig. 1). No alterations in parasite morphology have been observed during treatment; however, in malignant tertian infections only immature parasites are detectable in peripheral blood. The immune γ-globulin appears to be effective both against *Plasmodium falciparum* and *P. malariae* (Fig. 2), but probably has no action against gametocytes (Fig. 3).

Two children aged 2–3 years failed to respond to the injection of 1·2 gm. γ-globulin. They had been admitted with pneumonia and measles respectively,

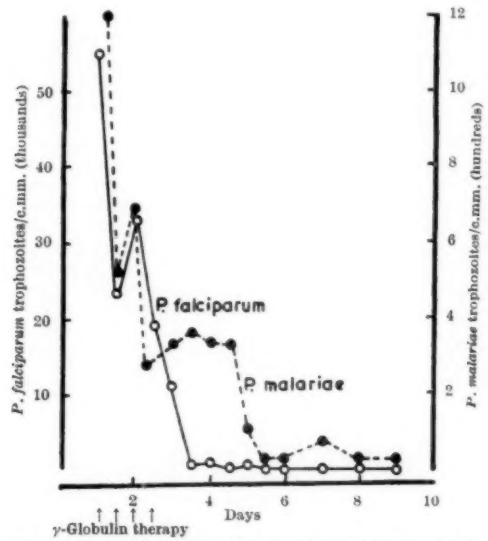

Fig. 2. Trophozoite counts of *Plasmodium falciparum* and *P. malariae* in a child with a mixed infection treated with immune γ-globulin; arrows show times of administration of γ-globulin by intra-muscular injection

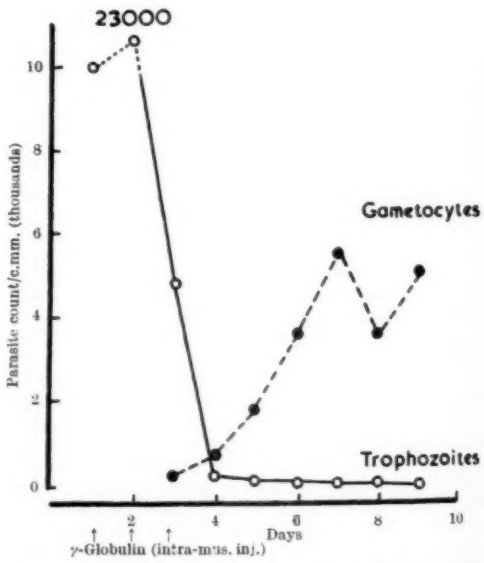

Fig. 3. Trophozoite and gametocyte counts in a child with acute *Plasmodium falciparum* malaria treated with immune γ-globulin. Arrows show time of administration of γ-globulin by intra-muscular injection

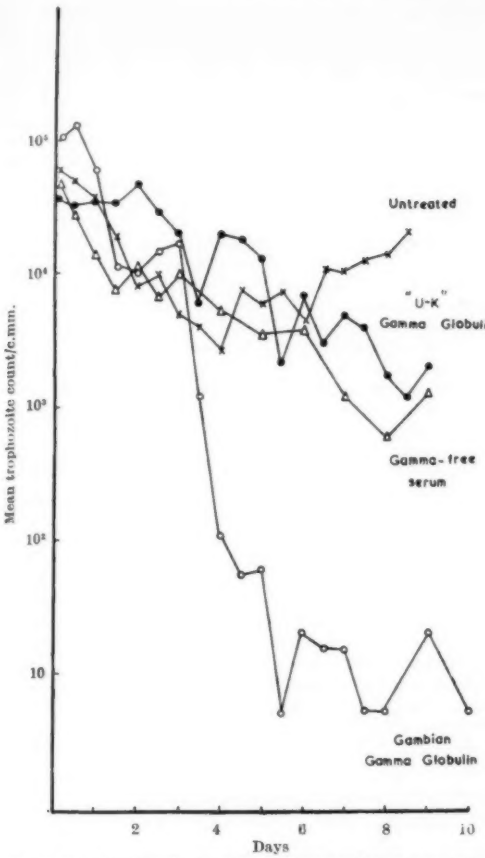

Fig. 4. Mean trophozoite counts (logarithmic scale) in four groups of children with acute *Plasmodium falciparum* malaria. The treated children received either (i) γ-globulin prepared from blood donors in Great Britain, or (ii) serum prepared from Gambian adults and containing 7 per cent 7S γ-globulin and 2 per cent 19S γ-globulin, or (iii) γ-globulin prepared from Gambian adults

and in both *P. falciparum* parasitæmia of relatively low density (8,000 and 16,000/c.mm.) was an incidental finding. Failure in these cases was probably due to inadequate dosage since 3 children of

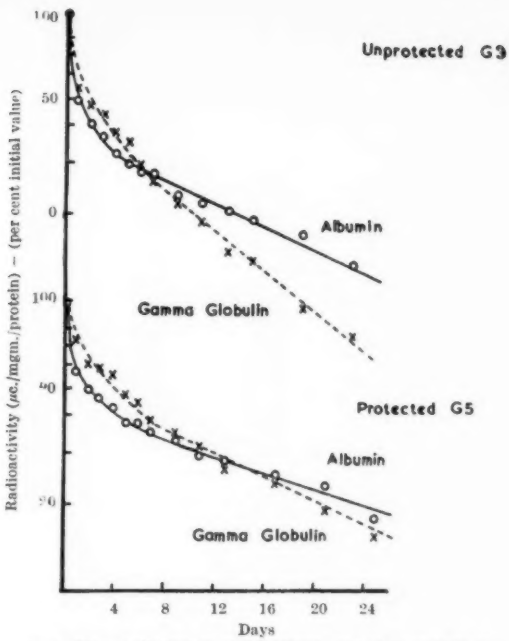

Fig. 5. Plasma elimination of ^{131}I-albumin and ^{125}I-γ-globulin in an unprotected adult Gambian and in a similar subject who had received 'Pyrimethamine' (25 mgm./week) for 6 years

comparable weight (10–12 kgm.) responded to the injection of 1·8–2·5 gm. γ-globulin.

In further experiments, 7 children with acute *P. falciparum* malaria received the γ-free fraction of Gambian serum (0·8–1·4 gm.). A single batch was used in all experiments and no child has developed signs of serum hepatitis. In addition, 4 children received γ-globulin prepared from the sera of blood donors in Great Britain (Lister Institute, Elstree, Herts.) and 2 were kept under observation without anti-malarial therapy. Trophozoite counts usually decreased in all these children. However, the response was irregular, and between the fourth and tenth days the mean trophozoite counts were considerably greater than those observed after treatment with adult African γ-globulin (Fig. 4).

Table 1. Turnover Data (Mean and Range) in Adult Subjects injected with Labelled Albumin and γ-Globulin

Subjects	No.	Total protein	gm. (per cent)	Intravascular pool (gm.)	Distribution ratio	Turnover-rate		
						Per cent I–V pool/d.	gm./d.	mgm./kgm./d.
Albumin								
Unprotected Gambians	4	8·1 (7·6–8·7)	3·6 (3·1–4·2)	86 (61–110)	1·3 (1·2–1·4)	10·8 (8·6–12·3)	9·1 (6·8–12·0)	170 (150–222)
Protected Gambians	5	8·3 (7·8–8·6)	4·1 (3·8–4·3)	91 (86–102)	1·2 (1·1–1·5)	10·5 (7·1–14·3)	9·4 (6·7–12·3)	176 (124–241)
W. Africans in Great Britain	4	8·6 (8·4–8·7)	4·6 (4·2–4·9)	113 (108–115)	1·3 (1·1–1·5)	10·4 (9·9–11·0)	11·7 (11·4–12·2)	159 (149–177)
Europeans in Great Britain (ref. 12)	11	7·8 (7·2–8·4)	4·5 (4·1–5·8)	123 (95–145)	1·4 (1·1–1·2)	10·4 (9·1–12·1)	12·7 (10·5–17·1)	185 (136–257)
γ-Globulin								
Unprotected Gambians	5	8·6 (8·3–9·2)	2·7 (2·2–2·9)	66 (57–82)	0·8 (0·7–1·0)	13·5 (11·8–15·7)	8·8 (7·9–9·7)	169 (139–203)
Pregnant unprotected Gambians	1	7·6	1·8	50	0·8	9·6	4·8	84
Protected Gambians	5	8·3 (7·8–8·6)	2·1 (1·5–2·4)	47 (30–67)	0·7 (0·6–0·8)	11·5 (8·5–15·4)	5·2 (4·6–5·7)	98 (80–113)
W. Africans in Great Britain	4	8·6 (8·4–8·7)	1·8 (1·6–2·0)	45 (38–55)	0·8 (0·7–0·9)	8·2 (7·8–9·3)	3·7 (2·7–4·3)	50 (42–57)
Europeans in Great Britain (ref. 11)	5		1·1 (1·0–1·1)	37 (28–44)	0·8 (0·6–0·9)	5·1 (4·0–5·7)	2·1 (1·5–2·4)	23 (18–28)

Distribution ratio = ratio extra- to intra-vascular protein mass

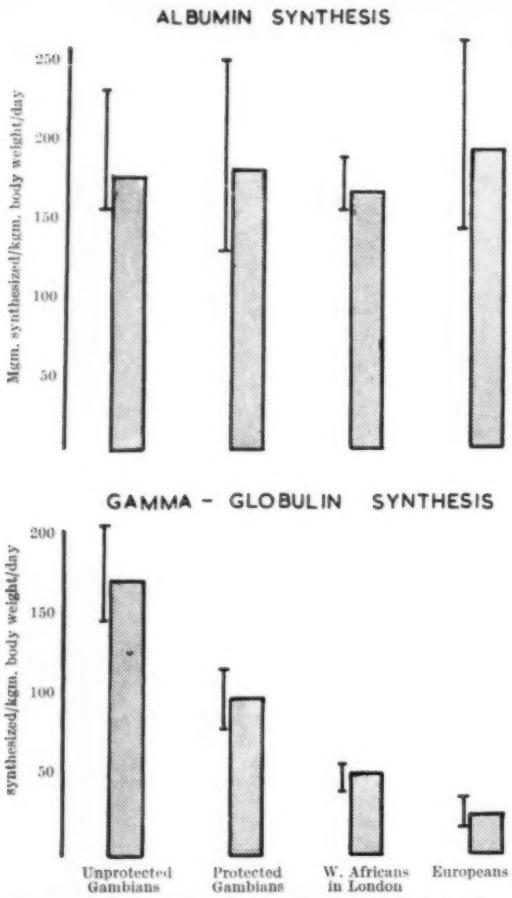

Fig. 6. Comparison of the average rates (and range) of albumin and γ-globulin synthesis in malarious and non-infected Africans and in healthy Europeans living in Great Britain

γ-Globulin Synthesis in Relation to Malarial Infection

The distribution and turnover of separate plasma protein fractions can be measured simultaneously by using two isotopes of iodine (iodine-131 and iodine-125) having different γ-emission spectra[11]. Using this method, rates of albumin and γ-globulin synthesis have been measured in apparently healthy Gambian adults exposed to malarial infection but not showing detectable parasitæmia. Similar studies were carried out on 5 Gambian adults who had received an anti-malarial prophylactic weekly for 4–5 years (Fig. 5) and also on 4 West African medical students who had suffered from malaria in childhood, but had been resident in England for 3–10 years. The experimental procedure has previously been described[11,12]; results were analysed according to the method of Matthews[13].

The rate of albumin synthesis was similar in the three groups of Africans and within the range for healthy Europeans. On the other hand, the rate of γ-globulin synthesis in unprotected Gambians was about 7 times that observed in Europeans; protection by anti-malarial therapy considerably reduced the rate of γ-globulin synthesis (Table 1 and Fig. 6). The rapid turnover of γ-globulin in unprotected adults cannot be attributed to the selective catabolism of isotopically labelled malarial antibody since γ-globulins prepared from European and adult Gambian donors have identical elimination-rates (Fig. 7). Considerably less γ-globulin was synthesized by an unprotected subject who was three months pregnant at the time of study (Table 1); this finding is of interest in relation to the increased susceptibility to malarial infection described in pregnant women living in hyperendemic areas[14,15].

West Africans resident for some years in the United Kingdom synthesized less γ-globulin than Gambian subjects maintained on anti-malarial therapy (Fig. 6); this difference must be attributed to environmental factors other than malarial infection. After several years in Great Britain, West Africans continue to synthesize γ-globulin at almost twice the rate observed in healthy Europeans; this may be due to environmental influences of early life or to genetically determined differences in γ-globulin metabolism.

Mechanism of Acquired Malarial Immunity

The preparation of γ-globulin from pooled sera of apparently hyperimmune donors and the use of susceptible children as recipients likely to have been exposed to homologous parasite strains, have provided optimum conditions for demonstrating the potency of naturally occurring malarial antibodies active against *Plasmodium falciparum* and probably also against *P. malariae*. The results show that the mechanism of acquired malarial immunity is basically similar to that observed with many other infections, being primarily dependent on the presence of protective antibodies which are associated with 7S γ-globulin. The response to immune γ-globulin

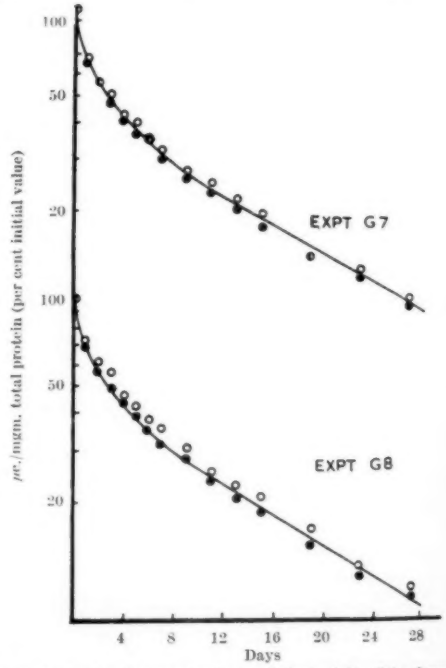

Fig. 7. Comparison in two unprotected African adults of the plasma elimination of γ-globulin prepared from European and Gambian donors and labelled with Iodine-125 and Iodine-131 respectively. ●, European γ-globulin; ○, Gambian γ-globulin

considered in relationship to the cycle of parasite development as well as the absence of morphological changes in the parasites of peripheral blood, suggest that antibody acts either on mature intracellular forms or on merozoites liberated from red blood cells. Protection is of limited duration and a treated subject was again susceptible three months after receiving γ-globulin. Other fractions of immune serum including 19S γ-globulin did not appear to have a protective effect nor did the γ-globulin of European blood donors.

These findings support the hypothesis that the relative resistance of infants born in hyperendemic areas is due to passive transfer of protective antibodies from the mother. This process may, however, be rendered relatively ineffective by partial loss of malarial immunity during pregnancy[14,15], which appears to be associated with a reduction in the rate of γ-globulin synthesis. Other factors, such as selective vector biting[16], deficiency of *p*-aminobenzoic acid in milk-fed infants[17,18] and the presence of a high proportion of fœtal hæmoglobin in circulating red cells[19] may contribute to the suppression of malarial infection during early life.

In areas of hyperendemic malaria, immunity is established only after long exposure to intense infection. This slow response is probably attributable both to the inherently poor antigenicity of the parasite and to the serological diversity of naturally occurring plasmodia. Acquired immunity is associated with hypergammaglobulinæmia[20,21] and with a striking increase in the rate of γ-globulin synthesis. Protection of Gambian adults against malaria over a period of several years reduced the average daily synthesis of γ-globulin by 3·5 gm., which is more than the total daily production in healthy Europeans. Since plasmodial infection leads to the production of complement-fixing antibodies[22] and precipitins[23], neither of which is species specific, it is likely that a considerable part of the γ-globulin synthesized in response to malaria is not protective antibody.

We wish to thank Sir Charles Harington, whose interest led to the initiation of this work, and Prof. R. R. Porter for much helpful discussion. We are grateful to Dr. D. Harling for clinical supervision of patients, Dr. W. d'A. Maycock for supplies of U.K. γ-globulin and Miss Margaret Polley for serological estimations of 19S γ-globulin. One of us (S. C.) is in receipt of a Medical Research Council grant.

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MOISTURE EXPANSION RELATIONSHIPS FOR A FIRED KAOLINITE-HYDROUS MICA-QUARTZ CLAY

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IN carrying out a detailed study of the moisture expansion characteristics of a clay containing 30 per cent kaolinite (poorly organized), 30 per cent hydrous mica (poorly organized) of the following approximate composition:

$$[Si_{6\cdot81}Al_{1\cdot20}]\ [Al_{2\cdot42}Fe_{1\cdot16}Mg_{0\cdot04}]$$
$$[Ca_{0\cdot30}K_{0\cdot25}Na_{0\cdot15}H_2O_{1\cdot42}]\ O_{20}(OH)_4$$

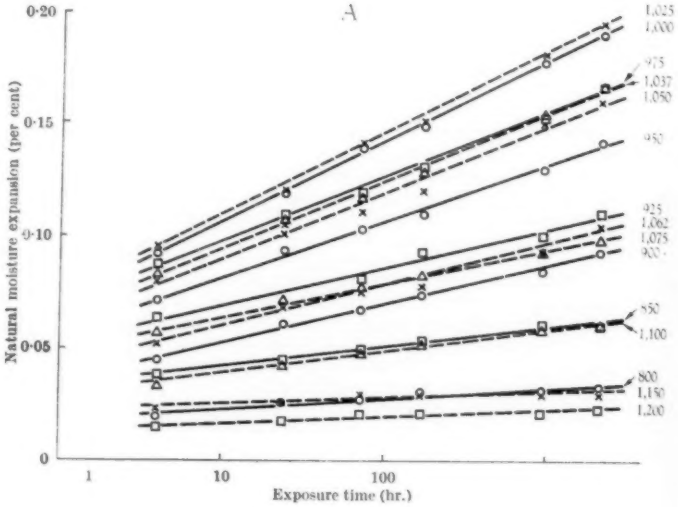
35 per cent quartz, 2 per cent hæmatite, and 1 per cent anatase, I have established relationships that might be found useful in interpreting the results from other clays and clay products.

The curves of moisture expansion versus firing temperature for specimens exposed to air for 90 days, and for specimens autoclaved at 220 lb./sq. in. for 2, 8, 30 and 200 hr. (measurements beginning from the same datum; specimens removed from the muffle at 300° C. and cooled for 2 hr. in a dry desiccator before initial measurement) show that the autoclave expansion curves parallel the natural expansion curve and that at a firing temperature of about 1,000° C. all have a maximum in expansion. If, however, autoclave expansion is plotted directly against natural expansion (Fig. 1), non-linear relationships exist over the firing range 950–1,200° C. for all periods of autoclaving, and the points below and above 1,000° C. separate into two curves for 30 and 200 hr. of autoclaving. (Linear relationships exist if autoclave expansion is plotted against the log of natural expansion.) The non-linear relationship expresses the fact that for this clay the ratio of autoclave expansion to natural expansion varies widely with firing temperature, and implies that there must be several reactive components produced over the range of firing temperature covered by the specimens. Mineralogical analysis has established that these reactive components are amorphous matter, glass, and anhydrous clay minerals. Amorphous matter, which is responsible for the coincident moisture expansion peak at about 1,000° C. in autoclaved and naturally exposed specimens, is equally reactive to water vapour in the air or to steam under pressure,

but anhydrous clay minerals and glass are far more active to steam than to water vapour in the air. Thus, for fired clay samples containing varying amounts of amorphous matter, anhydrous clay minerals, and glass, non-linear relationships must arise between autoclave expansion and natural expansion. Furthermore, the nature of the anhydrous clay minerals and of the glass will determine whether the autoclave and natural moisture expansion peaks occur at the same temperature.

It is well known that both natural moisture expansion[1,2] and autoclave moisture expansion[2–5] increase rapidly at first but in the later stages the increases are much slower. The interesting feature of the new results is that a plot of natural expansion against logarithm of time (Fig. 2*A*) is linear for all firing temperatures from 800 to 1,200° C. and exposure times from 4 hr. to 90 days. A plot of the autoclave expansions against the log of the time of autoclaving (Fig. 2*B*) shows also that a linear relationship exists for specimens fired at 1,000–1,150° C. for autoclave times for 2–200 hr. Similar relationships can be found from the data in the literature[2], if plots are made against log time rather than against time. However, data on other clays from the Division[6] suggest that at times longer than those investigated here the logarithmic relationship breaks down and that the complete curve of expansion against logarithm of time is frequently *S*-shaped. Consequently caution is needed in using the log-time or any other time relationship in predicting future values of moisture expansion from known results.

Weight measurements made at the same time as expansion measurements show that as the temperature of the natural moisture expansion peak is approached from low firing temperatures, progressively less water is required to produce unit expansion, but that at temperatures beyond that of the moisture expansion peak the same weight of water produces the same amount of expansion. The same is true for specimens expanded in the autoclave. Furthermore, the amounts of water per unit expansion for specimens fired above the temperature of the moisture expansion peak are much the same for the natural (1·52 per cent increase in weight for a 1 per cent increase in length) and autoclave (1·92 per cent increase in weight for a 1 per cent increase in length) expansion, that is, the moisture absorbed in the two processes must be held in the same way. However, there is a small amount of



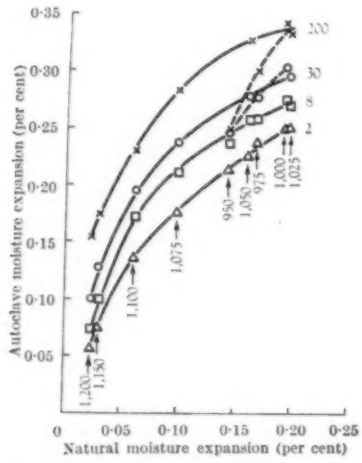


Fig. 1. Autoclave moisture expansion on a dry basis after 2, 8, 30 and 200 hr. of autoclaving versus natural expansion after 90 days of exposure for specimens fired at 950–1,200° C. The results for specimens autoclaved for 30 and 200 hr. after being fired at 950, 975 and 1,000° C. form separate curves which are shown by dotted lines

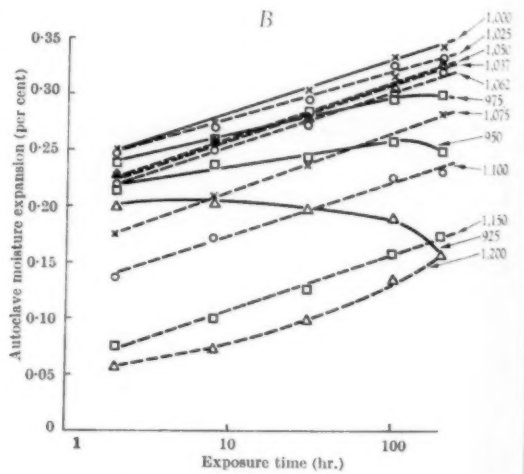


Fig. 2. *A*. Natural moisture expansion for specimens fired at 800–1,200° C. plotted against the logarithm of time of standing in air. Data for temperatures of 1,000° C. or less are represented by full lines, data for other temperature by dotted lines
B. Autoclave moisture expansion after drying at 110° C. for specimens fired at 925–1,200° C. plotted against the logarithm of the time of autoclaving. Data for temperatures of 1,000° C. or less are represented by full lines, data for other temperatures by dotted lines

water taken up in the autoclave which must be presumed to cause no expansion at all.

When specimens were desorbed up to a temperature of 500° C. by re-heating, they lost weight and contracted, but at about this temperature those that were low-fired (600–900° C.) showed an expansion with loss in weight which was not reversed until much higher temperatures of re-heating were reached. The magnitude of the expansion depended on the original temperature of firing and was only zero at a firing temperature of 950° C., that is, when the anhydrous mica was destroyed. Thus the expansion can be associated with the dehydration of the mica mineral regenerated from the anhydrous form by water sorbed after firing.

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VISUAL EFFECTS OF NON-REDUNDANT STIMULATION

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OPTICAL fields modulated randomly with respect to space and time ('visual noise') appear to offer a powerful new tool for the investigation of visual perceptual processes. In earlier work[1,2] I have described the use of such 'non-redundant' stimuli to shock-excite the visual system during the inspection of regular spatially repetitive patterns, so as to reveal the 'complementary image' that is generated in the visual system by such patterns. The present article is intended as an interim report on a wider range of new visual phenomena revealed by the same technique. It is not concerned with exposure to random fields as a form of 'sensory deprivation', which has interesting effects in a different category[3].

A typical source of 'visual noise' is offered by a de-tuned television receiver with high gain. Most readers will be familiar with the 'snowstorm' effect thus produced on the screen. An alternative, which gives greater flexibility[4] is to make up a ciné film-loop from successive samples of a random distribution of white (or black) spots. (Photographs of sandpaper, or sprinkled confetti, are remarkably adequate). With any such source, the following effects may readily be observed:

(1) *Discontinuity between foveal and peripheral fields.* The normal impression first created on inspection of visual noise is that of a homogeneous field of 'particles' in violent agitation. Individual 'particles' can be followed for a second or more in pseudo-Brownian motion. On prolonged inspection, however (especially if monocular), the foveal area of the field is clearly distinguishable from the remainder. Its motion is more coherent, as of a liquid boiling up from a restricted central source, and its average amplitude of pulsation is greater than that of the peripheral field. As the gaze is directed to different regions of the random stimulus, the 'boiling' foveal area can be clearly identified by the fact that it travels with the eye.

(2) *Monocular versus binocular viewing.* Considerable theoretical interest attaches to the contrast between monocular and binocular excitation. Most subjects spontaneously report that the monocular field appears the more densely populated, and scintillates more rapidly. With two eyes the field is seen as less 'sparkling', the motion more 'oily', the 'particles' fewer and more persistent. On most theories of binocular fusion it would not have been expected that the simultaneous and identical excitation of corresponding retinal areas should reduce the apparent density and frequency of what is perceived.

(3) *Effect of intensity.* One is led to ask whether simply halving or doubling the intensity of stimulation could produce the same effect as changing from binocular to monocular viewing or vice versa. But in fact rather the opposite is the case. At weak intensities the field appears much more 'oily' and is traversed by wriggling, interlocking snake-like shadow-patterns that may each extend over much of its area. At the normal intensity of a television screen, however, a change by a factor of 2 (or more) makes little difference. Binocular fusion here involves clearly more than the central summation of retinal impulses from 'corresponding points'. This is in line with the discovery by Jung[5] and others of separate cortical representations of each retina (in the cat).

(4) *Steady illumination in one eye.* It might be suggested that the occlusion of one eye causes centrifugal feedback, to both retinæ, which enhances the sensitivity of the unoccluded eye. As a partial test of this hypothesis the unused eye can be flooded with uniform illumination adjustable in brightness above or below the average level of the random source. The effect, however, is negligible, and not at all comparable with that of using both eyes to view the source.

(5) *The 'frame-adhesion' effect.* One of the most pleasing effects occurs when a portion of the random field is 'framed' within a movable contour such as the outline of a loop of wire. When the wire frame is moved and followed with the eye, the whole assemblage of 'Brownian particles' within it (and those immediately outside) are unmistakably seen to move with it. If the motion of the frame is rapid and oscillatory, the particles show a kind of inertia, lagging behind and swirling just as if immersed in a fluid.

When, however, a stationary object such as a finger-tip is placed within the framed area, practically the whole is suddenly stabilized, 'adhering' to the finger-tip. If the finger in turn is moved, it appears to be 'pursued' by a swarm of particles.

That the interactions concerned are central, rather than retinal, can be seen by presenting the noise to one eye and the frame to the other, when the effects are much the same, apart from a somewhat more noticeable time-lag in the coupling between frame and noise field.

XUM

(6) *Complementary images.* As already reported[1] the effects of superimposing random noise on spatially repetitive patterns are especially striking. When a source of noise is viewed through a transparency such as Fig. 1, the otherwise chaotic disposition of the 'particles' is at once organized into one or several superimposed geometrical figures (the 'complementary image' (ref. 1)) the principal directions of which run at right angles to the lines of the repetitive pattern. A similar but transient 'reorganization' is seen if the random source is viewed alone, immediately after inspection of the repetitive pattern.

The phenomenon is extremely tolerant of the optical definition of the random source. Even with a thin sheet of paper between the pattern and the television screen, the complementary image is clearly visible. An impression of a typical figure complementary to the stimulus of Fig. 1 is shown in Fig. 2. If the stimulus figure is a 'grid' formed by superposing sets of parallel lines at right angles, the complementary image lines run diagonally.

The complementary figures so generated are generally seen in motion. With Fig. 1, for example, the subject typically reports two or more superimposed 'rosettes' of the type of Fig. 2, which may be seen as contra-rotating, or all rotating clockwise or anticlockwise, more or less at will. If Fig. 1 is itself rotated, the complementary image rotates in the opposite direction.

When Fig. 3 is used, a petalloid radial complementary image is seen, with strong radial streaming along the petals. In addition, however, there is a slow and steady rotation of the noise field, with an angular velocity, varying with the radius, of approximately one radian a second. This turns out to be a quite distinct phenomenon, not dependent on spatial repetitiveness; for if visual noise is viewed through even a single annular aperture, it is still seen to rotate in each direction alternately.

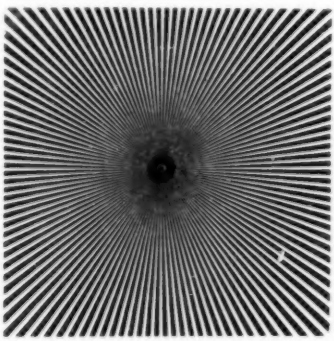

Fig. 1

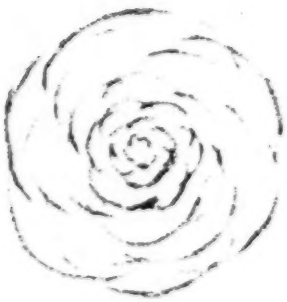

Fig. 2

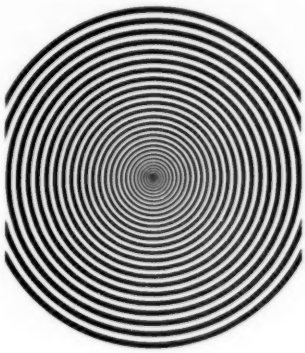

Fig. 3

(7) *Implications.* It is not my purpose here to advance interpretations of the phenomena described, on which we are working at present; but if they demonstrate anything clearly, it would seem to be the strong synthetic element in perception, which is present even when 'associations' in the usual sense might be expected to be absent. What is seen under excitation by random noise bears only an approximate relation to the stimulus. Instead, even against a blank background, it seems to be a fairly strongly structured synthetic 'imitation'—that is, a distribution of activity matched presumably in respect of certain statistical features, but sufficiently well structured for changes in its position to be readily attributable and easily perceived (Exp. 5). With a background of a regularly periodic nature, the structure seen bears still less resemblance to that of the stimulus.

The foregoing are only samples of the new data revealed by this way of 'shock-exciting' the visual system, which will be discussed more fully elsewhere. The present article is intended mainly to direct attention to the power and potential value of this class of stimulus. In communication engineering, random noise is often used as a 'neutral' test-signal to reveal the characteristic modes of response (resonances and the like) of an information-system. In the study of the visual system it seems clear from the foregoing examples that visual 'noise' offers a tool with a corresponding ability to uncover some characteristic modes of response of perceptual mechanisms. Formally, it can be regarded as the neutral member of a family of stimuli at the extremes of which are stroboscopic illumination (spatially uniform, temporally periodic) and our parallel-line patterns (temporally uniform, spatially periodic). At each of these extremes characteristic responses are also evoked[1,6]; and it can scarcely be doubted that all three classes of phenomenon have something to contribute to our theorizing about perceptual processes.

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LETTERS TO THE EDITORS

ASTROPHYSICS

Presence and Correlation of Fine Structure in Jovian Decametric Radiation

JUPITER'S non-thermal emission in the decameter region of the spectrum takes the form of sporadic radio noise storms having durations ranging from minutes to several hours[1]. Observers agree that the storms show fine structure, in part composed of bursts which typically have durations ranging from several tenths of a second to several seconds. However, there has not been agreement as to the presence of still finer structure or the intrinsic origin of any of the fine structure.

In 1956 Kraus[2] and Gallet[3] reported very short bursts having durations of tens of m.sec. or less, which they associated with Jupiter, but other observers[4–7] have been unable to confirm this. As for the origin of fine structure, radio sources of angular size much greater than Jupiter often show pronounced ionospheric scintillation with components occasionally as short as several seconds[8,9]; the Jupiter source must be expected to show at least similar effects. Recordings made by Gardner and Shain[4] on three nights in 1956, using receivers spaced 25 km. east–west, indicated little correlation of the noise fine structure over this base-line, the lack of correlation suggesting that ionospheric irregularities might account for much if not all of the burst structure. On the other hand, a spaced-receiver observation by us in 1958 (ref. 10), while confirming the existence of considerable amplitude distortion, showed 80 per cent correlation in the time of arrival of bursts at stations separated by as much as 30 miles east–west. Florida–Chile observations in 1960 by A. G. Smith and Carr[11] also indicated partial correlation over a very long base-line, suggesting the reality of some intrinsic fine structure.

To resolve these questions a new series of base-line correlation observations was initiated by us in March 1961. Four identical crystal-controlled total-power receivers working at 22·200 Mc./s. with 6 kc./s. band-width were fed with identical antennas. The locations chosen were the Yale Observatory's Bethany Observing Station (central site), Hendrie Hall at New Haven (15 km. south of Bethany), Van Vleck Observatory at Middletown (30 km. east of Bethany), and Pomfret School (100 km. east of Bethany). The audio outputs of the receivers were transmitted to Bethany on telephone lines. During Jupiter storms all four channels plus timing marks were recorded on a high-speed photographic recorder with a time constant of 30 msec. Records obtained on 19 nights up to August 1 exhibited various degrees of correlation, including 6 nights on which correlation was essentially perfect, without amplitude distortion. Correlation tended to improve through the Jupiter observing season from March onwards, suggesting that the phase with respect to Jovian opposition (July) or to the terrestrial season (summer) may be significant.

A small portion of a storm with correlated events (July 18) is reproduced in Fig. 1 (the New Haven receiver was not functioning during this run). Storms with similar portions of closely correlated events were observed on June 5 and July 11, 20, 27, and 30. No form of ionospheric effect is known that preserves its correlation over 100 km.; accordingly we conclude that fine structure such as that of Fig. 1 is characteristic either of the source at Jupiter or of the interplanetary medium, but that this intrinsic structure is often largely obliterated by local ionospheric effects.

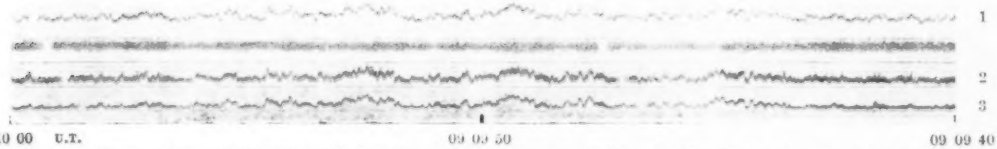

Fig. 1. A 20-sec. portion of the high-speed recording of the Jupiter storm of July 18, 1961, showing close correlation of fine-structure events received at Middletown (3) and Pomfret (2) stations separated by 30 and 100 km. respectively from the Bethany Station (1)

During this observing season we noted the presence of numerous correlated fine-structure components having durations of hundredths rather than tenths of seconds. In comparison, our observations during 1960 showed no events having durations less than 0·1 sec. We must regard this extreme fine structure as real, in view of its correlation over long base-line and association with Jupiter storms (not present before or after). Thus the occasional existence of Jupiter bursts of extremely short duration as reported by Kraus and Gallet seems to be confirmed.

The co-operation and assistance particularly of E. Milone of Yale Observatory, Dr. F. Zabriskie of Van Vleck Observatory, J. McCullough of Pomfret School, and financial support from the National Science Foundation are gratefully acknowledged.

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GEOPHYSICS

Confirmation of Outer Atmospheric Asymmetry postulated to explain the False Zodiacal Light

In 1952 and afterwards[1-3], I suggested that the false zodiacal light—an early morning pyramid of light above the western horizon, resembling the zodiacal light which at that time would be on the opposite side of the sky—might be explained if Earth's gaseous tail were driven off mainly from the western twilight rim of the globe. I can now point to some recent findings that support this idea[4,5].

A terrestrial gaseous tail has been repeatedly postulated (by Lomonosov in 1753, Houzeau in 1888, Evershed in 1902, Astapovich[6], Fesenkov[6], Divari[6], and many others) to explain the counterglow or gegenschein, an antisolar luminosity with a variable brightness and a gaseous emission spectrum (Karimov[6], Tikhov), which Soviet parallax measurements place at an apparent distance of ~ 20 Earth radii. The gases forming such a tail could only be of atmospheric origin; presumably solar radiation pressure carries off atoms or molecules thermally expelled in high trajectories from the underlying denser atmosphere. I pictured "the solar heating steadily unwinding a ribbon of gas from the upper atmosphere into the ecliptic plane" (ref. 2, p. xviii).

The false zodiacal light, observed and named by Divari and Fesenkov in 1946 (ref. 6, p. 22) but previously described by Sykes[7] and by Schmid[7], is presumably a luminosity in the lower part of the tail, below the counterglow. The counterglow luminescence is thought to be excited by solar corpuscular emission in the region beyond the terrestrial magnetosphere (Astapovich[6]). To-day one may suspect that the nearer luminosity of the false zodiacal light is connected with the van Allen radiation, though other theories are possible[1,2,6].

Most remarkable is the asymmetry of the false zodiacal light: it is seen in the western sky as a post-midnight opening-out of the counterglow ellipse, but it is never seen in the eastern sky. If the gaseous tail itself is asymmetric, the reasons could be that (*a*) the rotation of Earth assists the solar radiation pressure at the evening twilight rim of Earth, but opposes it at the morning twilight rim; (*b*) at the evening twilight rim the atmosphere has been heated by the Sun, so that the supply of thermally accelerated exospheric particles, that is, the density of the exterior atmosphere, would here be significantly greater than on the morning rim (ref. 2, p. xvi).

Point (*b*) is now confirmed by the exospheric densities calculated from the orbital behaviour of artificial satellites. Beginning at ~ 200 km. height above the terrestrial ellipsoid, there is a minimum of density at 6 hr. true local time and a maximum at 14 hr. The difference between maximum and minimum steadily increases with altitude, and at 1,700 km., according to the model used by Römer[8], it exceeds two orders of magnitude. At 18 hr. the pressure remains high; at 700 km. height the supply of gas particles is ~ 7·5 times greater on the evening rim than on the morning rim of Earth (ref. 4, Fig. 4); at 1,700 km. it is ~ 12 times greater (ref. 5, Fig. 2).

The exterior atmosphere is chiefly maintained by solar energy absorbed and stored at lower (*F*-layer) levels. This explains the lag of the density maximum toward the afternoon and the continuing higher density in the evening.

The false zodiacal light, seen only in the west, must not be confused with the zodiacal twilight[8,9]—the atmospheric component of the ordinary zodiacal light—which according to Divari's specific finding appears with equal intensity on the western and eastern horizons. Moreover the zodiacal twilight is concentrated toward the horizon, while the false zodiacal light, opening out from the counterglow (Divari[6], Fig. 10), is strong at higher angles of elevation.

It is also obvious that the false zodiacal light, extending from the counterglow, is unrelated to the cosmic dust-cloud component of the ordinary zodiacal light. Aside from the nearness, the variability and the emission spectrum of the counterglow as demonstrated by the Soviet authors, there is Roach and Rees's demonstration[10] that the counterglow and the cosmic zodiacal light lie in slightly different planes. The former is, as expected, in the ecliptic, while the cosmic dust cloud is symmetrical with a plane through the Sun determined mainly by Jupiter's gravitation[11]. (Long-lasting confusion has been caused by the reports of those who profess themselves unable to find the slightest asymmetry or variability of the zodiacal light with respect to the ecliptic. These reports may be dismissed as pointless, because there is no imaginable reason why the zodiacal dust cloud around the Sun should lie precisely in the ecliptic, which is merely the orbital plane of the Earth.)

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BIOPHYSICS

Extendability of Tendons undergoing Contraction–Relaxation

Equilibrium melting of tendons[1] and collagenous tissues[2] follows the theoretical behaviour expected for a first-order phase transition. Thus, the ordered crystalline regions of polypeptide chains disintegrate into randomly oriented coils of the amorphous phase. Rat-tail tendons subjected to melting by lyotropic agents undergo rapid contraction followed by relaxation to rupture, and these physical events are easily measured characteristics of the melting process. Stress-strain curves of thermally shrunk tendons have been shown to obey equilibrium elastic theory for amorphous polymers[3]; for example, stress is proportional to $(a - a^{-2})$, where a is the strain. This communication deals, however, with a kinetic analysis of melting of rat-tail tendons in concentrated aqueous urea at pH 7·0 and 37° C.

When the velocity of length-time observations (Fig. 1) was analysed kinetically, equation 1 was found to agree with the data. The terms involved are dL/dt, the velocity of change of length at any time (t); $(dL/dt)_c$, the initial velocity of contraction; $(dL/dt)_r$, the terminal velocity of relaxation; and K_{cr}, the specific rate-constant:

$$\text{Log}\,[dL/dt - (dL/dt)_r] = \log\,[(dL/dt)_c - (dL/dt)_r] - K_{cr}t \quad (1)$$

Temperature had a pronounced influence on the rate process, but it systematically followed equation 2:

$$\text{Log}\,K_{cr} = A + B/T \quad (2)$$

The resistance to disruption of ageing tendons in 10 M urea solution, furthermore, increased according to equation 3:

$$\text{Log (time-to-break)} = C + D(\text{age, or weight of animal}) \quad (3)$$

Generally, it is believed that the attack on collagen by urea breaks stabilizing hydrogen bonds[4]. A complication in the selectiveness of this protein–urea interaction is that a condensation can occur between free amino groups of the protein with a slight amount of ammonium cyanate which exists in equilibrium with aqueous urea[5]. This should not, however, interfere with hydrogen bond rupture; in fact it could promote it by assisting to liberate one conjugor (amine side-chains of lysine or hydroxylysine) from a previously existing hydrogen bond with those groups. It has also been shown that aqueous urea condenses with long hydrocarbon side-chains to form 'adduct' compounds[6]. Since collagen in tendons has few such long hydrocarbon side-chains, it does not appear that adduction of urea presents any great complication.

Accepting that action of urea results in rupture of secondary, tertiary, and higher-order —OH stabilizing bridges[4], we investigated next how this depolymerization influenced tendon elasticity. To answer this question experiments were conducted in which the

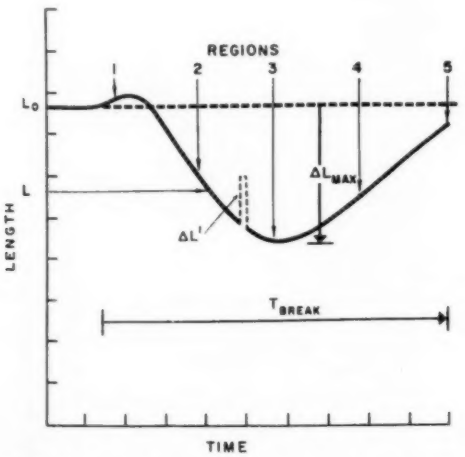

Fig. 1. Changes in length of rat-tail tendon produced by 10 M urea, pH 7·0 at 37° C. The five characteristic regions are: (1) initial lengthening of 'dry' tendon as solution is added, (2) period of contraction, (3) point of maximum-contraction, (4) period of relaxation, and (5) rupture. Initial length L_0, actual length L, maximum contraction L_{max}, and time-to-break T_B are also clearly identified. The dotted line displacement ($\Delta L'$) is the periodically induced momentary-strain

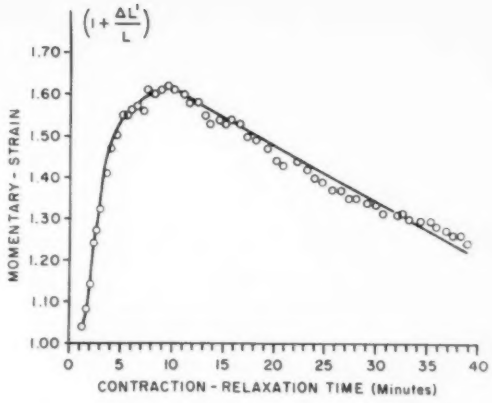

Fig. 2. Dependence of momentary-strain $\left(1 + \frac{\Delta L'}{L}\right)$ on time of contraction–relaxation

momentary extension of a tendon was measured by adding and removing a weight. A change in length ($\Delta L'$), illustrated in Fig. 1, was determined periodically and superimposed on the contraction–relaxation sequence. Fig. 2 shows a plot of strain ($1 + \Delta L'/L$) as a function of contraction–relaxation time.

Figs. 1 and 2 reveal that a tendon becomes more easily extendable as a result of the action of urea which also produces shortening and re-extension. Maximum extendability occurs near the point of maximum contraction, and it decreases with subsequent lengthening of the tendon. It is obvious, therefore, that extendability and contraction-relaxation are inversely related.

The change of strain with time $\frac{d}{dt}(\Delta L'/L)$ was evaluated and plotted as a function of time. Numerous graphs of these data suggested that there was a semi-logarithmic relationship between velocity of strain and time. A final graph was made of the difference between velocity of strain at time (t) and that constant velocity of strain which characterized the relaxation period. A linear relationship was observed with this function, and equation 4 was derived on the basis of these numerical analyses:

$$\text{Log}\left[\frac{d}{dt}(\Delta L'/L) - \frac{d}{dt}(\Delta L'/L)_r\right] = \log\left[\frac{d}{dt}(\Delta L'/L)_c - \frac{d}{dt}(\Delta L'/L)_r\right] - K'_{cr}t \quad (4)$$

These measurements of the rate of increased tendon extendability during contraction support the interpretation that the latter reflects a crystalline/amorphous phase transition, or melting. It is not clear, however, why extendability and re-extension are inversely related because the tendon is continuing to degrade to the point of rupture. A maximum degree of shortening would appear to be theoretically possible, but this may not be realized due to possible independence of $(dL/dt)_c$ and $(dL/dt)_r$. Consideration of factors responsible for incomplete shortening and relaxation might lead to a better understanding of the kinetic mechanism of contraction–relaxation. Finally, this could give a more precise explanation of effects of ageing on rate of tissue dissolution such as shown by equation 3.

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METALLURGY

New Ternary *R*-Phases with Silicon

The *R*-phase was first found[1] in the chromium–molybdenum–cobalt ternary system. Its crystal structure has been determined by Shoemaker *et al.*[2]. Additional *R*-phases were later found in the molybdenum–manganese–cobalt and molybdenum–manganese–iron ternary systems[3], as well as in the titanium–manganese binary system[4]. Recently, in the investigation of several ternary systems with silicon a number of new *R*-phases have been identified. Table 1 gives the approximate composition and the lattice parameters of these new *R*-phases:

Table 1. Composition (approximately) and Lattice Parameters of New *R*-Phases

Alloy	Annealing temperature (° C.)	c (Å.)	a (Å.)	c/a
$Ti_6Mn_{7\cdot5}Si_{13}$	1,000	19·23	10·87	1·77
$Nb_6Mn_{7\cdot5}Si_{14}$	1,000	19·28	10·89	1·77
$Ta_6Mn_{7\cdot5}Si_{14}$	1,000	19·19	10·86	1·77
$Mo_6Mn_{7\cdot5}Si_{10}$	1,000	19·18	10·85	1·77
$W_6Mn_{7\cdot5}Si_{10}$	1,000	19·22	10·86	1·77
$V_{37}Fe_{41}Si_{22}$	1,100	19·23	10·79	1·78
$V_{41}Co_{40}Si_{19}$	1,100	19·14	10·78	1·78
$V_{41}Ni_{40}Si_{19}$	1,100	19·10	10·82	1·77

It is interesting to note that in all alloys as yet known to consist of the *R*-phase one of the component elements has a relatively large co-ordination number 12 atomic radius in relation to that of the others. For example, in all the *R*-phases listed in Table 1, the ratio of the co-ordination number 12 radii of the two transition elements lies between 1·07 and 1·12. In the case of the ternary *R*-phases with manganese and silicon the concentration of the element with the large atomic radius needed in order to stabilize the *R*-phase may be quite small, for example, 2 *a*/*o* titanium, 5 *a*/*o* niobium or tantalum, 3 *a*/*o* molybdenum or tungsten. However, the amount of vanadium required in the ternary systems with iron, cobalt or nickel and silicon is very much larger.

It has now been established that the unidentified phases previously designated as I, III and V co-existing with the σ-phase in the vanadium–iron–silicon, vanadium–cobalt–silicon and vanadium–nickel–silicon systems[5] are identical with the corresponding *R*-phases listed above. Previously unidentified phases II and IV also co-existing with the σ-phase in the vanadium–iron–silicon and vanadium–cobalt–silicon systems have now been identified as having the α-Mn structure. An α-Mn phase also occurs in the vanadium–nickel–silicon system. Details of these phase relations will be published.

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α-Manganese Phases containing Technetium-99

The existence of α-manganese phases in the zirconium–technetium, niobium–technetium and molybdenum–technetium systems was recently reported by V. B. Compton *et al.*[1]. During the course of our investigation of alloy phases involving technetium-99 with transition metals, α-manganese phases were found in the binary systems scandium–technetium, titanium–technetium, hafnium–technetium and tantalum–technetium. Pertinent information is given in Table 1. The alloys were made by arc-melting transition metals of high purity with technetium-99. X-ray data were obtained from specimens in the as-cast condition with a powder camera of 114·6 mm. diameter using filtered copper radiation.

Table 1. Lattice Parameters of several α-Manganese Phases containing Technetium-99

System	X-ray data: Alloy composition, atomic per cent	a_0 (Å.)
Sc–Tc	87·5 Tc; 12·5 Sc	9·509 ± 0·001
Ti–Tc	87·5 Tc; 12·5 Ti	9·579 ± 0·001
Hf–Tc	87·5 Tc; 12·5 Hf	9·603 ± 0·001
Ta–Tc	83·3 Tc; 16·7 Ta	9·565 ± 0·001

Crystal structure and lattice dimensions of technetium-99 have already been reported by R. C. L. Mooney[2], using a microgram specimen. He found a hexagonal close-packed structure with $a_0 = 2{\cdot}735 \pm 0{\cdot}001$ Å., $c_0 = 4{\cdot}388 \pm 0{\cdot}001$ Å., $c/a = 1{\cdot}604$. In the present investigation lattice dimensions of technetium-99 were obtained from an annealed needle of high-purity technetium-metal. The dimensions are as follows: $a_0 = 2{\cdot}743 \pm 0{\cdot}001$ Å., $c_0 = 4{\cdot}400 \pm 0{\cdot}001$ Å., and $c/a = 1{\cdot}604$. Since Mooney reported a slight impurity in his mixed sample, the lattice parameters reported here are probably more accurate.

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A Cold Crucible for High-Temperature Melting Processes

EXPLORATORY research in the solid-state field has resulted in the development of a new melting technique for the processing of silicon. Although this method has been associated primarily with the purification of silicon and its growth as single crystals, it has important applications in the more general field of metallurgy.

In our earlier work[1], a container was made from a silver tube by pressing a longitudinal dent into its surface for most of the length. This silver 'boat' was internally water-cooled and contained a silicon bar in the depression. When power was applied to a short radio-frequency coil surrounding a small part of the boat, a narrow molten zone was formed in the bar. This zone was traversed along the bar to zone-refine the silicon and leave only the impurity boron, which does not segregate appreciably in silicon. The silver boat acts as a radio-frequency transformer, and current is induced into the silicon bar from the silver underneath it, as well as from the primary coil above it. The liquid silicon does not wet the cold silver surface; it should be emphasized, however, that melting is complete, without any skin or shell of solid silicon.

The results of pinch effect caused by mutually opposing electric currents can be observed following changes in the power setting or movements of the coil. Partial levitation of the melt can be obtained with suitably designed coils, particularly at a frequency of 500 kc./s. or lower; but at frequencies of 2 Mc./s. and above, these magnetic effects are very much reduced.

Another form of horizontal boat which has certain advantages in special cases can be made by using several straight parallel tubes mounted in a semicircle and closely spaced, so that the melt is confined in the boat by its surface tension.

The growth of silicon single crystals has been accomplished in the past by pulling from silica crucibles or by means of a crucible-free method (see, for example, ref. 2). The methods suffer from some disadvantages; in the former method, oxygen is introduced into the growing crystals due to chemical reaction between molten silicon and silica; in the latter procedure the density of dislocations tends to be high, and the diameter of the crystal is limited in size. Some attempts were made to grow single crystals horizontally in the silver boat, but these failed mainly on account of the large temperature gradient at the solid/liquid growing interface.

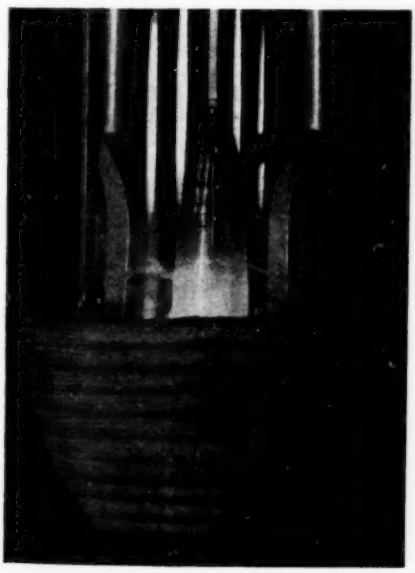

Fig. 1. A silicon single crystal grown from a cooled silver crucible

In order to obtain a more favourable thermal symmetry and a correct temperature distribution for the growth of single crystals, several novel types of crucible[3] have been designed. In one particular form (Fig. 1) a number of tapered and water-cooled silver tubes is arranged vertically, and curved inward at the bottom to join at their narrow ends and form a basket. This crucible is water-cooled internally, and mounted inside a radio-frequency primary coil which has the shape of an upturned skep. Power is induced through the spaces between the tubes, and also by induction due to the current which flows circumferentially in each tube. The tubes are placed sufficiently close together to prevent the melt, when formed, from escaping. Again it is important to note that melting is complete, and that no thin skin of silicon remains as in 'skull melting'.

Single crystals of silicon which are oxygen-free and dislocation-free have been grown by the Czochralski method from crucibles of this and similar types, using silicon which was obtained by the thermal decomposition of pure silane gas[4]. Life-times up to 1 msec. have been measured and resistivities greater than 1,000 ohm cm. have been preserved. Electrical and radiochemical tests have not detected the presence of silver in these crystals.

The cold-metal crucible has been also successfully used as a non-contaminating container for the melting and processing of such materials as ferrites and allied oxides, rare earth metals, molybdenum, titanium, uranium and boron; it is not restricted to high-temperature melting applications only.

We wish to acknowledge the work of Mr. F. J. Raymond, who is a co-inventor, and also other assistance given by colleagues of Standard Telecommunications Laboratories, Ltd., who are associated with this project. We thank the management of these Laboratories for permission to publish this communication.

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RADIOCHEMISTRY

Organic Extraction from Fused Uranium Chlorides

FUSED plutonium and uranium chlorides may be a good nuclear fuel for a fast breeder reactor[1-4]. The aim of the present work is to investigate some chemical properties of fused chloride components, in particular, the presence of dissociated or associated or non-ionic molecules, and the possibility of using the liquid salt–liquid organic extraction. Some recent results concerning the extraction processes with fused

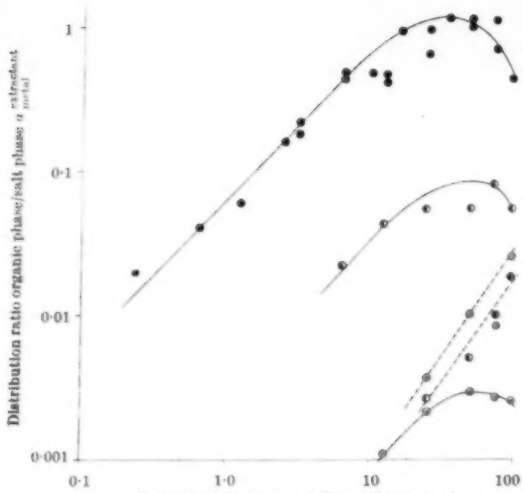

Fig. 1. ●, Uranium; ◐, copper; ○, potassium ——, TOA; – – –, TBP

salts are presented in ref. 5. In the present investigation the salt phase contains: diluent (nuclear non-active): eutectic mixture of KCl–CuCl, 1 : 2 molar ratio, and fissionable material: uranium tetrachloride (prepared by the reaction, uranium dioxide + carbon tetrachloride at 450° C.) with a concentration of uranium tetrachloride of 1·5 per cent w/w. The salts were dried under vacuum by heating and fusing in the presence of dry hydrochloric acid and dry argon, at a temperature of 180° C. The organic phase contains: diphenyl as diluent and one of the following extractants: tri-*n*-octylamine (TOA), tri-*n*-butylphosphate (TBP), di-*n*-butylphosphoric acid (HDBP); dried in vacuum by heating. All components of fused salts were used as labelled atoms: copper-64, potassium-42, chlorine-36, uranium enriched, registered by suitable radiometry α, β, γ. The extraction process was carried out in a small volume, 1·6 ml., in 180° C., for 30 min. (15 min. with shaking): after separation of both phases (using different solidification temperature) they are dissolved: salt phase in nitric acid solution, organic phase in toluene or benzene. All operations were performed in glove boxes with dried atmosphere. The distribution ratio $\alpha_{metal}^{extractant}$ for each metal is measured as ratio of total radioactivity of organic phase to salt phase (the volume ratio of the two phases is approximately 1). The results are shown in Fig. 1.

The experimental distribution ratios are as follows:

uranium: $\alpha_U^{TBP} \geqslant \alpha_U^{HDBP} \geqslant \alpha_U^{TOA}$

copper: $\alpha_{Cu}^{TOA} \gg \alpha_{Cu}^{TBP} > \alpha_{Cu}^{HDBP}$

potassium: $\alpha_K^{TBP} > \alpha_K^{TOA}$

TOA: $\alpha_U^{TOA} \gg \alpha_{Cu}^{TOA} \gg \alpha_K^{TOA}$

TBP: $\alpha_U^{TBP} \gg \alpha_K^{TBP} > \alpha_{Cu}^{TBP}$

From these results, it may be concluded that: in fused chlorides there are cations K^+ and Cu^+; non-ionic molecules, CuCl Cu_2Cl_2 and potassium chloride; anions, $Cu_2Cl_3^-$, UCl_5^-.

For the CuCl–TOA extraction system, with a small concentration of TOA: 0·1 $M \leqslant$ concentration TOA $\leqslant$ 0·3 M, the ratio of molar concentration, copper/TOA in the organic phase is about 1 : 3, which corresponds to the formula $[TOACu^+]\,[Cu_2Cl_3^-]$. It also seems that for uranium tetrachloride–TOA extraction the extractable species is an ion-pair, namely, $[TOACu^+]\,[UCl_5^-]$.

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A Simple Microdiffusion Technique for the Radioiodination of Proteins

A number of methods for the radioiodination of proteins and amino-acids have been described[1,2]. Some have relied on transfer of elemental iodine from an oxidizing medium by extraction with an organic solvent; in others oxidation of iodide to iodine has been effected in the presence of the material to be iodinated with the associated risk of damage to the latter by the oxidizing reagent in the mixture.

Many of these techniques are somewhat cumbersome; moreover, some are such that little confidence can be felt either in the purity and integrity of the product or in the safety of the experimenter.

Faced with the task of labelling insulin with iodine-131 at high specific activity (~ 100 mc./mgm.) we have used Conway's microdiffusion technique[3], using in the first instance No. 2*a* diffusion units and more recently modified cells of smaller (~ 1 cm.). diameter. The technique has also been successfully applied to the radioiodination of beta- and gamma-globulins, albumin and tyrosine.

The material to be iodinated, dissolved in 0·2 *M* borate buffer (*p*H 8·0), is placed in the central chamber of the unit; radioiodine diluted with the desired amount of inactive carrier is placed in the outer compartment together with an appropriate volume of acidified dichromate. Diffusion of iodine vapour into the central compartment is largely complete in a matter of 2 hr. (80–90 per cent is a typical recovery in most cases) although the exposure may be terminated at any desired time by addition of excess alkali to the outer chamber. Adequate distribution of iodine through the protein solution is effected either by agitation of the unit or by use of a small magnetic stirrer in the central compartment.

The maximum permissible volume of the oxidizing mixture is critically dependent on the weight of carrier iodine available for transfer. Where μgm. quantities of protein are to be iodinated, adequate transfer-rates of the μgm. amounts of iodine usually desired in these circumstances can only be obtained using oxidizing mixture volumes of a few microlitres. This places a lower limit on the amounts of protein of less than 5 μgm. which may conveniently be used, and hence indirectly on the specific activities which may be obtained with tolerable starting amounts of iodine-131. Nevertheless, specific activities of the order of 100 mc./mgm. insulin can readily be achieved using about 3–5 mc. iodine-131.

Radiochemical purity of the iodinated protein has been checked by electrophoresis and, in the case of insulin, by chromatography in a secondary butanol/

acetic acid solvent system[4]. No loss of biological activity of the iodinated insulin was observed using the rat epididymal fat pad bioassay technique.

Overall recovery of radioactivity in the final product has been of the order of 25 per cent. Though this implies considerable wastage of iodine-131, it has been felt that the advantages in this method of the mildest possible oxidative environment for the protein and the minimal radiation damage to which the latter is exposed compared with other techniques merit its application in many circumstances.

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CHEMISTRY

Pyrolysis and Gas Chromatography for the Detection of the Benzene Ring in Organic Compounds

In a systematic investigation concerning the correlation between structure and pyrolysis products, it was found that pyrolysis of compounds eluted from gas chromatographic columns could be used for the detection of the benzene ring when present in organic compounds.

A conventional gas chromatograph was used in the experiments. The top of the column was provided with a heated quartz tube filled with 'Chromosorb' (about 2 gm.). Silicon oil was used as stationary phase.

The quartz tube was connected to a short thick-walled glass capillary with silicone rubber tubing. The capillary was closed with a serum-cap and the carrier gas was led into the column through a side tube just behind the serum-cap. The quartz pyrolysis tube was surrounded by a small furnace of which the temperature was measured with a thermocouple between the walls of the furnace and the pyrolysis tube. The compound under investigation was injected through the serum-cap in the usual way on to the hot 'Chromosorb'. Of liquid compounds 1 μl. was injected. Of solid compounds 5–10 μl. of a solution of the compound in methanol was used.

Some 100 different aromatic compounds containing a benzene ring were tested, and experiments with benzyl alcohol, benzylbenzoate and benzyl phenylacetate are shown in Fig. 1 as characteristic examples. It is seen that two peaks are present in the chromatograms. One peak was identified by infra-red spectroscopy as benzene, the other as toluene. This behaviour is characteristic for compounds that contain the benzene ring and a side-chain or groups such as $—OCH_3$ or $—OOCH_3$. The formation of toluene in these cases may be explained on the basis of free radical mechanism in which methyl radicals are formed from the $—OCH_3$ and $—OOCH_3$ groups respectively. This is made evident by the following experiment. When a saturated solution of benzoic acid in methanol is pyrolysed a small toluene peak is observed following a large benzene peak. When, however, a saturated solution of benzoic acid in chloroform instead of methanol is pyrolysed no toluene peak is found. A small peak with the correct retention time of chlorobenzene now appears next to benzene. For the compounds tested the ratio of the peak areas is a typical constant for the compound which is pyrolysed when the experimental conditions are kept identical (Table 1).

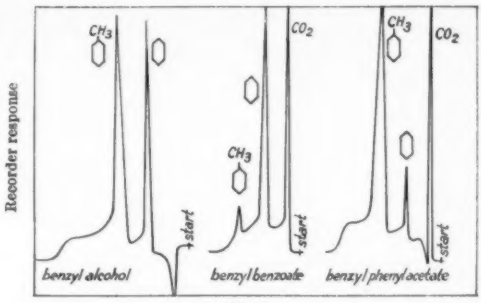

Fig. 1. Chromatograms of pyrolysis products of benzyl alcohol, benzyl benzoate and benzyl phenylacetate

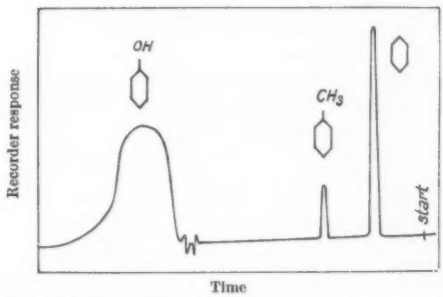

Fig. 2. Chromatogram of the pyrolysis products of anisole

The result of the pyrolysis of anisole is shown in Fig. 2. The formation of phenols was always observed in the pyrolysis of compounds containing the C_6H_5O-group such as salicylic acid (pyrolysed in methanol solution), *p*-cresylacetate, anisole and others.

The formation of benzene and simple benzene derivatives on pyrolysis of aromatic compounds was thus found to be a very general reaction for these compounds. Although it is known that benzene can be synthesized from simple aliphatic compounds by pyrolysis, no benzene peaks were observed when aliphatic compounds were tested under normal conditions. Aromatization clearly is negligible under the conditions used.

During the experiments recorded here separate series of tests on minute amounts of simpler organic

Table 1. Ratio of Peak Areas at Pyrolysis Temperature 550° C.

Compound	Area of toluene peak / Area of benzene peak
Toluene	7·5
Toluene	6·9
Toluene	7·9
Benzyl alcohol	1·7
Benzyl alcohol	1·7
Benzyl alcohol	1·8
Benzyl phenylacetate	5·4
Benzyl phenylacetate	4·9
Benzyl benzoate	*c.* 0·12
Benzyl benzoate	*c.* 0·12
Anisole	*c.* 0·127
Dioctyl phthalate	*c.* 0·08

compounds such as aliphatic alcohols, amines, acids and esters were pyrolysed and analysed in the way described. From all these compounds characteristic chromatograms of the pyrolysis products were obtained which made it possible to differentiate between compound groups, individual members of homologous series and between isomers such as normal- and iso-alcohols.

It is believed that a valuable technique for identification of the small amounts of material eluting from gas chromatographic columns can be based on this method of pyrolysis.

The method does not seem to be restricted to volatile compounds[1].

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A Coating with Antifouling Properties from the Reaction between Cuprous Oxide and Sodium Silicate

Films prepared from dispersions of zinc dust in sodium silica media of a high silica ratio of about $Na_2O : SiO_2/1 : 3{\cdot}8$ by weight (Pass, A., and Meason, M. J. F., private communication) have been reported to become substantially insoluble in water within a few hours without the aid of curing agents previously needed to produce satisfactory air-drying zinc silicate paints[1]. The mechanism of the formation of these films is only partially understood, and in the course of work undertaken to elucidate it, changes in viscosity occurring when zinc and other pigments are dispersed in silicate media are being examined.

Various pigments were dispersed in aqueous solutions containing 16·6 per cent of sodium silicate having an $Na_2O : SiO_2$ ratio of $1 : 3{\cdot}55$ by weight, and the changes in viscosity of the dispersions with time were measured at a shear-rate of 54 sec.$^{-1}$ using a Couette co-axial cylinder type viscometer.

Fig. 1 shows curves which illustrate the viscosity/time relationships characteristic of the two main groups of pigments which were studied. Dispersions of non-reactive pigments such as talc, micaceous hæmatite, titanium dioxide and iron oxide decrease in viscosity with time due to an improvement in dispersion brought about by the action of the viscometer. Films produced from these dispersions will only become insoluble in water on long exposure in air or by the application of heat or curing agents. In contrast, the viscosity of dispersions of some other pigments including zinc dust, cuprous oxide and mercuric oxide increases gradually with time evidently because the pigments react with or cause a reaction in the medium. Of particular interest was the indication that, as with zinc, films of cuprous oxide in sodium silicate media of a high silica ratio might cure unaided at room temperature and this has been confirmed by experiment.

The proportion of cuprous oxide in the dispersion and the $Na_2O : SiO_2$ ratio in the medium can be varied over quite a wide range, and the film will still become insoluble in water within, at most, an overnight drying period. Methyl cellulose, bentonite, micronized mica and similar viscosity control agents can be included to improve the application and film-forming properties of the dispersion. A typical composition having satisfactory film forming and curing

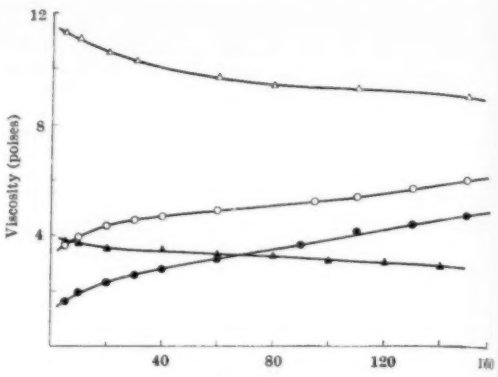

Fig. 1. Viscosity at constant shear-rate as a function of time. △, Talc; ○, cuprous oxide; ●, zinc dust; ▲, micaceous hæmatite. The quantities of pigment added to 120 ml. of sodium silicate solution were 56 gm., 225 gm., 450 gm. and 300 gm. respectively

properties is made by dispersing 225 parts by weight of cuprous oxide and one part of methyl cellulose in 140 parts of an aqueous solution containing 18·6 per cent of sodium silicate having an $Na_2O : SiO_2$ ratio of $1 : 3{\cdot}8$.

Films of this composition and of variants of it have shown no fouling during a period of 95 weeks immersion in Sydney Harbour, Australia. The most abundant macrofouling organisms recorded at this severe exposure site are: *Balanus* (*Balanus*) *amphitrite* var. *cirratus* Darwin; *Bugula avicularia* L., *Bugula neritina* L.; *Hydroides norvegica* Gunnerus, *Spirorbis* sp.; *Watersipora cucullata* (Busk), *Conopeum reticulum* (Linne); *Mytilus planulatus* Lamarck and *Crassostrea commercialis* Iredale and Roughley, and settlement of at least three of these species took place on inert control panels during each month of the immersion period (ref. 2 and Wisely, B., private communication). The average fouling-free life of 46 commercial paints immersed along with the silicate compositions was 26 weeks (Laurie, H. A., private communication).

There are indications that the inclusion of zinc dust up to the extent of about 10 per cent of the cuprous oxide content of such dispersions may prolong the fouling-free life of the film by depressing the solution-rate of the copper in the early stages of immersion.

We thank the Department of the Navy, Australia, for the use of its immersion facilities in Sydney Harbour and Mr. H. A. Laurie for the periodic examination of panels. The work forms part of a programme sponsored by the Department of the Navy, and this communication is published by permission of the Chief Scientist, Department of Supply, Australian Defence Scientific Service, Melbourne, Victoria, Australia.

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Evolution of Hydrocarbons from Impure Titanium Getters

When titanium getters are heated and evaporated in vacuum hydrocarbons are formed from carbon and hydrogen impurities in the metal[1]. The evolution of hydrocarbon gases from two grades of titanium has been investigated in this laboratory with an 'Omegatron' mass spectrometer and found to be related to the partial pressure of hydrogen in the system and the carbon impurity in the getter.

Two grades of titanium were used having the following chemical analyses and termed here grade *A*: 0·055 per cent carbon, 0·0065 per cent hydrogen and grade *B*: 0·007 per cent carbon, 0·016 per cent hydrogen. Getter filaments were made by twisting together a titanium wire of 1 mm. diameter and 5 cm. long with three tungsten wires each of 0·5 mm. diameter. The getter filaments were mounted within glass cylinders (8·5 cm. diam. × 8 cm. high) inside the stainless steel vessel of a bakeable high-vacuum plant. The vessel was exhausted by a diffusion pump charged with silicone 704 and fitted with a liquid-nitrogen trap. All the vacuum seals were made with metal gaskets and after baking the system an ultimate pressure of 10^{-8} torr was obtained. To raise the partial pressures of the gases evolved by the getter, and thereby aid their detection by the 'Omegatron' spectrometer, the speed of pumping the vessel was limited to 10 l./sec. by a diaphragm in the mouth of the liquid-nitrogen trap.

When either grade of titanium was evaporated in high vacuum the principal hydrocarbon gas formed had a mass of 15 (CH_3). However, when the titanium was volatilized in hydrogen at a pressure of 10^{-7}–10^{-6} torr (nitrogen equivalent) the predominant hydrocarbon formed had a mass of 16 corresponding to the saturated compound of methane. Subsequent experiments were then made with hydrogen leaked into the vessel during pre-heating of the getter, evaporation of titanium and after film deposition. Mass spectra were recorded of the atmosphere in the system during each of these three stages. A typical experiment was completed in a total time of 70 min. with a pre-heating time of 40 min. and an evaporation time of 15 min.

To ensure that impurities in the tungsten heater did not influence the experiments bare tungsten filaments were heated in the same manner as titanium-loaded filaments, and the resulting mass spectra

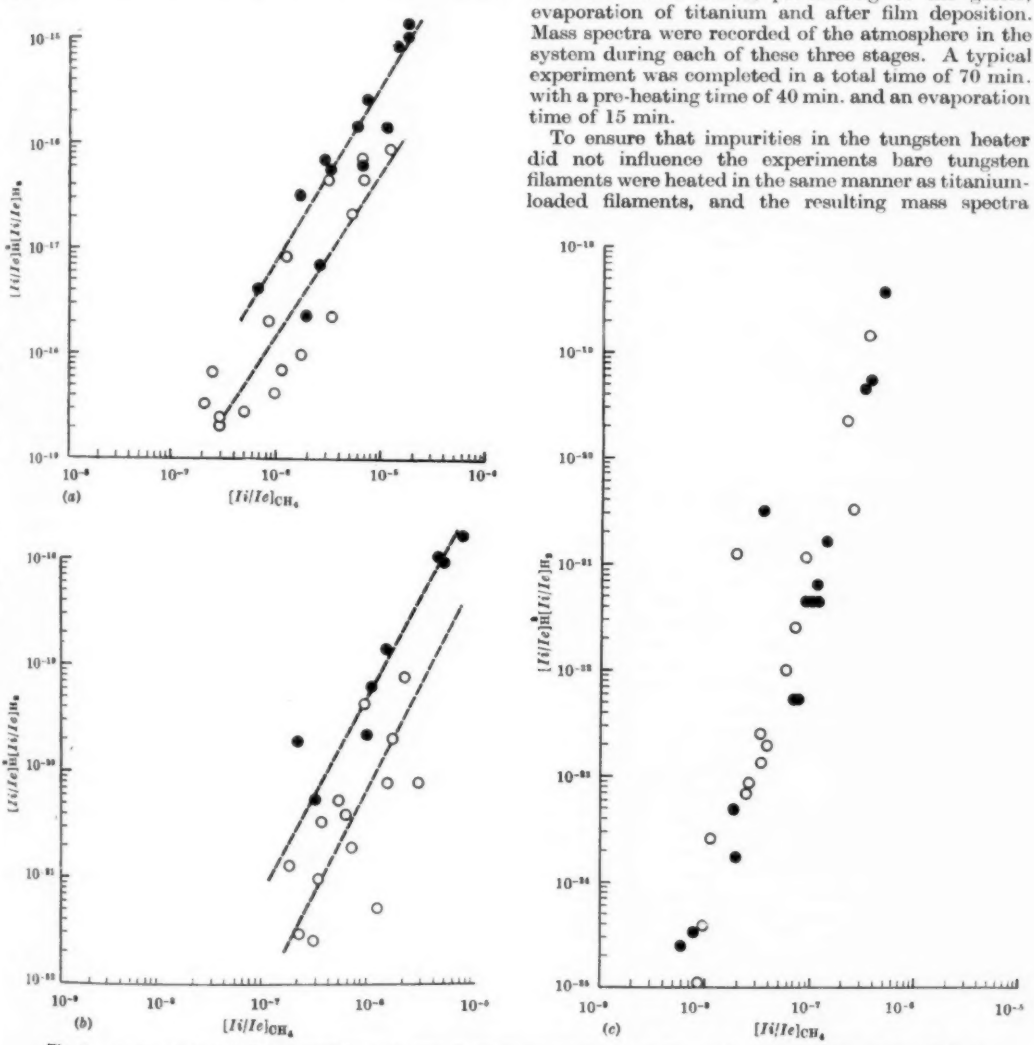

Fig. 1. *a*: ○, Type *A*, before evaporation; ●, type *B*, before evaporation. *b*: ○, Type *A*, during evaporation; ● type *B*, during evaporation. *c*: ○, type *A*, after evaporation; ●, type *B*, after evaporation. $[Ti/Ie]$ is the ratio of ion to electron current

showed that negligible amounts of hydrocarbon gas were evolved.

To find the relation between the partial pressures of hydrogen and hydrocarbon gas in the titanium experiments, the peak heights of atomic hydrogen, diatomic hydrogen and methane in the mass spectra were measured. Corrections were made to the measured values for variations in rate of evaporation during film growth and for changes in hydrogen concentration during the recording of the mass-spectra. The hydrocarbon evolution during the pre-heating and evaporation of the getter indicated that it followed the law of mass action if one assumed a simple equation of the kind: $C + 2H + H_2 \rightleftharpoons CH_4$. Thus by plotting methane concentration $[CH_4]$ as a function of $[H^2][H_2]$, Figs. 1*a* and 1*b* were obtained for the pre-heating and evaporation stages respectively and empirical lines drawn through the points showed an approximately linear relation between $[CH_4]$ and $[H^2][H_2]$. The plotted values are divided into two groups in each graph, dependent on the carbon content of the getter metal. The partial pressure of hydrocarbon gas measured during the post-evaporation stage (Fig. 1*c*) did not show a dependence on the carbon impurity in the titanium. Also, the curve following the values in Fig. 1*c* departs more markedly from a linear relation between $[CH_4]$ and $[H^2][H_2]$ and the mass action law is not considered to apply.

During pre-heating the reversible chemical reaction between carbon and hydrogen must occur at the surface of the hot titanium. However, when evaporation begins it is uncertain whether the reaction can still proceed at the high filament temperature ($\geqslant$ 1,625° C.). It is possible that the reversible reaction now occurs at the surface of the condensing titanium film. At the pressure of operation ($\simeq 3 \times 10^{-7}$ torr) hydrogen molecules strike the titanium film at a rate of $4{\cdot}5 \times 10^{14}$ molecules sec.$^{-1}$ cm.$^{-2}$ and rough calculations of the rates of arrival of titanium atoms give values between 1·5 and $5{\cdot}0 \times 10^{15}$ atoms sec.$^{-1}$ cm.$^{-2}$. Thus the ratio of impinging titanium atoms to hydrogen molecules varies from 3 : 1 to 10 : 1. The heat released at the surface by the condensing titanium and the heat radiation from the getter filament may both be responsible for a reversible reaction at the getter surface.

Although there are many aspects of the foregoing work which are unsatisfactory, for example, the sole consideration of methane production ignoring other hydrocarbon species, it does show that metal film getters will only be completely effective in the sorption of hydrogen if they are prepared free from carbon.

Acknowledgments are made to Mr. Elwell, Division chief analyst of Imperial Chemicals Industry, Metals Division, and to Mr. Webb, chief analyst of Johnson Matthey, for their kind assistance in the chemical analysis of the titanium used in these experiments. The experimental work reported here was sponsored by C. T. R. Division, Atomic Energy Research Establishment, Harwell.

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Detection of Carboxyl Groups in Conjugation with the Porphyrin Ring System

The band positions in the visible region of a porphyrin spectrum are influenced by the electrophilic nature of the substituents conjugated with the porphyrin ring system: the more electrophilic the substituent the farther the absorption maxima lie towards longer wave-lengths. Thus, when a reagent reacts specifically with a particular group to produce a derivative which has significantly different electrophilic characteristics, a corresponding change in the spectrum is observed, and this change indicates the presence of the group in question. For example, porphyrins with carbonyl groups conjugated with the ring system react with hydroxylamine to produce oximes which have their absorption maxima at wave-lengths shorter than those of the original carbonyl compounds. Such a spectroscopic change indicates the presence of a carbonyl group in conjugation with the tetrapyrrolic ring system. Methods of this type, which require only a trace of material, have played an important part in the structural determination of naturally occurring porphyrins.

My recent work has required a similar procedure for the detection of a carboxyl group in conjugation with the porphyrin ring system. It has been argued that since the position of the absorption maxima of porphyrins is dependent on the electrophilic nature of their substituents, the visible spectrum of a carboxylic acid, when in conjugation with a tetrapyrrolic ring system, should be different from that of its anion. Since the spectra of porphyrins in alkaline solution are often ill-defined, it has been found advantageous to carry out the present investigation on a series of pyridine hæmochromes (ferrous complexes of porphyrins, co-ordinated through iron to pyridine). These derivatives have well-defined two-banded spectra in alkaline media, and the position of the longer wave-length band (α-band) is particularly sensitive to changes in the electrophilic nature of the particular substituent conjugated with the porphyrin ring system. However, hæmochromes are stable only in alkaline solutions, and to provide a spectrum which would approximate that of the un-ionized acid, the methyl ester was used instead of the free acid, which under the alkaline conditions would be ionized.

The pyridine hæmochromes have been prepared by dissolving the porphyrin iron complex in pyridine 0·1 *N* sodium hydroxide (1 : 1) and reducing the mixture with sodium dithionite. Table 1 records the α-band position of pyridine hæmochromes derived from porphyrins containing a conjugated carboxylic acid group and compares them with values obtained from the corresponding methyl esters. A difference of more than 10 mμ between these values is observed, which provides a reliable diagnostic test for the presence of a carboxylic group in conjugation with the tetrapyrrolic ring system.

Table 1

Compound	α-Band of pyridine hæmochrome (mμ)	
	Acid	Ester
2-Carboxyl-deuteroporphyrin *	549	563
2-Carboxyl-4-vinyl-deuteroporphyrin *	555	567
2,4-Dicarboxyl-deuteroporphyrin *	554	567
4-Acrylic acid-deuteroporphyrin (ref. 2)	561	572
Rhodoporphyrin (ref. 3)	553	566
Porphyrin *a* acid (ref. 4)	560	572

* These compounds were prepared by oxidation of the corresponding formyl porphyrin, using Jones's reagent (ref. 5).

Fischer and Orth[1] quote a value of 563 mμ for the α-band of a hæmochrome derived from rhodoporphyrin. In view of the values reported here their result appears anomalous and seems to approximate that of the ester rather than of the acid.

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Examination of Side-Chain Interactions in Poly-L-Tryptophan

While hypochromism has been examined quantitatively by Tinoco[1] for polynucleotides and Imahori and Tanaka[2] have reported decreased extinction of the peptide absorption band at 1900 Å. in poly-L-glutamic acid in the helical conformation, it was of interest to examine interactions of the aromatic side-chains of poly-L-tryptophan. The results of absorption and fluorescence studies of the polyamino-acid compared with a suitable model for the monomer are discussed in this communication.

Poly-L-tryptophan, having an average molecular weight of 17,800, was supplied by Dr. Michael Sela. Acetyltryptophan is commercially available and tryptophan ethyl ester was prepared by a modification of the method of Brenner and Huber[3]. Solvents were distilled prior to use except for dimethylformamide, which was 'Spectroquality' reagent purchased from Matheson, Coleman and Bell. Absorption spectra were taken with a Cary recording spectrophotometer model 14, and intensities of emitted light were measured with the Aminco-Bowman fluorometer with the monochrometers set at constant excitation and emission wave-lengths.

The limited solubility of polytryptophan did not permit examination over a wide range of solvent polarities. In this work the solvents used were dimethylformamide, dimethyl sulphoxide and *n*-butylamine/water (9 : 1 by vol.). Poly-L-tryptophan absorption obeys Beer's law in these solvents over the twenty-fold range of concentration studied. The absorption maxima and their extinction coefficients are shown in Table 1 together with the results of Hurd and Bauer[4] for polytryptophan (average molecular weight 4,000) in ethanol.

The total areas under the absorption curves as a measure of the oscillator strength, *f*, were determined after extrapolation below 2600 Å., where accurate measurements were not obtainable because of solvent absorption. The *f*'s are very nearly the same for acetyl- and poly-tryptophan in the solvents used in this investigation. Similarly, temperature variation between 25° C. and 80° C. had no exclusive effect on the absorption spectrum of the monomer or polymer.

Table 1. Absorption Maxima and Extinction Coefficients of *N*-Acetyl- and Poly-tryptophan

Solvent	*N*-Acetyltryptophan $\lambda_{max.}$ (Å.)	*N*-Acetyltryptophan $\varepsilon \times 10^{-3}$ *	Poly-L-tryptophan $\lambda_{max.}$ (Å.)	Poly-L-tryptophan $\varepsilon \times 10^{-3}$
Dimethylformamide	2830	5·99	2830	5·84
	2910	5·35	2910	5·13
Dimethyl sulphoxide	2835	5·68	2835	5·74
	2910	5·16	2910	5·15
n-Butylamine/water (9 : 1 by volume)	2840	5·58	2840	5·43
	2915	5·07	2915	5·10
Ethanol	2825 (ref. 5)	5·62	2820	5·49†
	2905	4·90	2905	4·68

* These values are the average of 10–15 determinations and are ± 0·05.
† Values from Hurd and Bauer (ref. 4) for polytryptophan, the average molecular weight of which is 4,000.

Although there are no significant differences between the absorption properties of acetyl- and poly-tryptophan, tryptophan–tryptophan interactions may be detected more readily by the fluorescence properties of the aromatic amino-acid side-chain. Emission intensities in the 3400 Å. region were measured for solutions having optical densities of 0·050–0·250 at the absorption maximum. In all solvents, the polymer was consistently 35–45 per cent less fluorescent than either acetyltryptophan or tryptophan ethyl ester in the same solvent. The decreased fluorescence of the polymer may be a reflexion of quenching as a consequence of the high local concentration of the aromatic groups.

The absence of hypo- or hyper-chromic effects in the absorption of polytryptophan suggests that either the transition moments of the freely rotating aromatic side-chains are not oriented or interactions between identical transitions cannot be detected by absorption studies. The present work also indicates that the high quantum yield of some tryptophan-containing proteins is not explicable in terms of tryptophan stacking as has been suggested[6]. Borrowing from or lending energy to other groups in the protein may account for unusual fluorescent properties of some albumins as recently reported by Weber[7].

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Absence of Correlation between Odour and Molecular Vibration

In 1928 Dyson[1] proposed a relationship between odour and molecular vibration. This hypothesis was worked out in more detail by Wright[2–6], who correlated the odour of a compound with its low-frequency vibrations (below 800 cm.$^{-1}$).

Wright postulates that odour-perception is caused by the interaction between the odorous molecule and a pigment molecule present in the olfactory cell. The former is attached to the latter by "forces of adsorption, or something of the sort"[5]. The pigment molecule is permanently in an excited electronic state and can only fall back to its electronic ground-state if the relevant selection rule is relaxed by a perturbation due to the odorous molecule in "a process akin to Fermi-resonance"[6]. This perturbation is thus assumed to occur only if the odorous molecule and the receptor molecule have one or more identical vibration frequencies. The electronic transition then triggers an electrical process in the cell membrane. Leaving the electronic transition and its selection rules out of discussion in this communication, we will now examine the vibrational interactions more closely.

It is easily seen that no correlation can be found between the vibrations of a free molecule and its odour if the molecule forms a complex with the receptor molecule, as the vibrational frequencies of

the complex are never the same as those of the constituent parts. As a consequence any molecule attached to the pigment molecule would give rise to odour-perception if the mechanism proposed by Wright applies.

A second possibility exists in very weak interactions, which, for example, give rise to coupling of vibrations of molecules in crystals. As the perturbation is weak, it is very questionable, however, whether it can change the selection rule for the electronic transition. The strongest objection to this vibrational coupling lies in the odours of hydrogen sulphide and deuterium sulphide. There are no grounds to believe that these compounds behave differently from all other substances. From the fact that the vibrational (and also rotational) frequencies of hydrogen sulphide and deuterium sulphide are completely different, it follows that there should be a distinct difference between the odours of these two compounds.

In an experiment similar to that described by Wright as inconclusive, the odours of hydrogen sulphide, deuterium oxide and deuterium sulphide were compared. The fundamental frequencies[7] of hydrogen sulphide, deuterium oxide and deuterium sulphide are listed in Table 1. As an influence of molecular rotations cannot be excluded, the rotational constants[8,9] are also given.

Table 1. The Fundamental Frequencies and Rotational Constants in cm.$^{-1}$ of Deuterium Sulphide (D_2S), Deuterium Oxide (D_2O) and Hydrogen Sulphide (H_2S)

	D_2S	D_2O	H_2S
ν_2	934	1,179	1,290
ν_1	1,892	2,666	2,611
ν_3	1,999	2,789	2,684
A	5·47	15·38	10·39
B	4·49	7·25	9·04
C	2·44	4·83	4·72

Judged from the vibrational frequencies, the odours of hydrogen sulphide and deuterium sulphide should be quite different; deuterium oxide should have an odour falling between that of hydrogen sulphide and deuterium sulphide. The rotational constants also indicate that the odours of hydrogen sulphide and deuterium sulphide would be different if molecular rotations play a part.

The odour of deuterium sulphide was tested in the following way. Deuterium sulphide was synthesized by adding anhydrous aluminium chloride to a solution of sodium sulphide in pure deuterium oxide. The trace of gas (the smell of which was near the threshold of detection) was kept in a stoppered bottle which was opened only long enough for each person to recognize the odour. As the deuterium in deuterium sulphide exchanges very rapidly, especially when in contact with the mucous membrane of the nose, this membrane was thoroughly 'deuterated' immediately before each experiment by spraying deuterium oxide into the nose, especially in the region of the olfactory epithelium. Recognition of the odour of *p*-dichlorobenzene before and after deuteration showed that this treatment does not affect the observer's sense of smell. The four observers all agreed on the following:

(1) Deuterium oxide is odourless. (2) Deuterium sulphide cannot be distinguished from hydrogen sulphide. (3) The intensity of the smell of deuterium sulphide is independent of the precautions taken to ensure that no hydrogen–deuterium exchange takes place. This proves that the actual odour of deuterium sulphide was smelt and not that of traces of hydrogen sulphide which may have been present.

This experiment thus seems to indicate that odour-perception is not linked with the vibrations of the free molecules.

If relaxation of the selection rules for an electronic transition is assumed to trigger the nerve impulse, it is very plausible that the formation of a weak complex with the accompanying redistribution of charge in the participating substances is the first step in the process of odour-perception. It is clear that there are other less-complicated processes, such as induction effects, which are able to trigger nerve impulses after complex-formation. Unfortunately little is known at present about the pigment molecules in the olfactory cells. It seems fruitful, however, to investigate the possibility of correlations between odour and molecular properties such as electron distribution.

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Large-Scale Polymer Column Fractionation

The gradient elution method for polymer fractionation was introduced by Williams[1–3]. To fractionate larger amounts of polymer, a scaled-up apparatus was necessary. Previous experience in column design in this laboratory advised against an increase in the dimensions of a single column to avoid difficulties in temperature control and channelling. Consequently, an apparatus was built with six parallel columns, each 1 m. in length and 2·5 cm. in inside diameter. A sketch of the multicolumn unit is shown in Fig. 1. The upper ends of the aluminium columns are welded to the bottom of a common solvent-mixing container. The metal container is 7 litres in capacity and is fitted with a heater, regulator, stirrer, and sight glass. The solvent mixture is heated to the proper temperature for the desired column temperature gradient. The lower ends of the columns are connected to a common outlet and immersed in a water thermostat. The temperature gradient along the insulated columns is found to be nearly linear, as measured by four evenly spaced thermocouples. The apparatus is charged and operated similarly to single columns previously described[1–3]. Two ganged 'Minipumps' (Milton Roy Company, Philadelphia, Pennsylvania), each with an adjustable 12–120 ml./hr. pumping capacity, regulate the rate of withdrawal of polymer solution from the common outlet. The pumps also replenish the solvent at the head of the column at the same rate, thus establishing the solvent gradient and degree of fractionation. The evaporation of the solvent for recovery of fractions is done automatically, *in situ*, using a rotary vacuum evaporator, fitted with a nitrogen inlet, vacuum line, and water-bath. A low evaporation temperature coupled with the nitrogen ensures polymer stability.

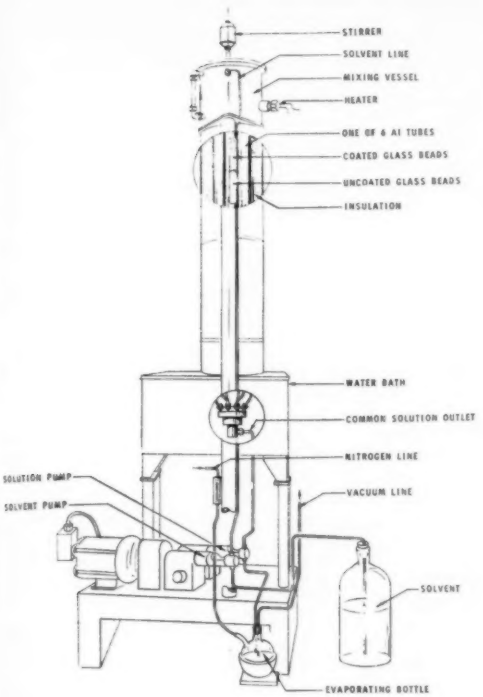

Fig. 1. Multi-column unit

As an example of apparatus performance, Table 1 gives results on the fractionation of 34 gm. of 'Vistanex *LM–MS*', a commercial polyisobutylene. The columns were packed with glass beads, average diameter 0·1 mm. The non-solvent employed was acetone, the solvent benzene. The temperature decrease down the column was from 50° to 28° C. At a throughput of 80 ml./hr., the fractionation required eight days. Polymer recovery was 95 per cent. The molecular weights were determined by viscosity measurements in carbon tetrachloride at 30° C. and evaluated according to the relation given by Fox and Flory[4]. Table 1 shows good fractionation in light of the low degree of original inhomogeneity; for the unfractionated polymer, $M_w/M_n = 2{\cdot}6$. As the apparatus requires little attention after starting up, fractionations require appreciably less effort than conventional precipitation methods and no more time than single-column operation. Successful fractionations have been made with sample sizes of 30–50 gm., depending on polymer molecular weight and type.

Table 1. Large-scale Fractionation Data for Polyisobutylene

Fraction	Gm.	Weight (per cent)	(η)	Molecular weight
A	0·3026	0·89	0·035	1,150
B	1·1217	3·28	0·058	2,500
C	1·6848	4·93	0·103	5,700
D	2·8666	8·38	0·155	10,300
E	2·8142	8·23	0·219	17,100
F	2·1895	6·40	0·233	18,800
G	2·0337	5·95	0·277	24,200
H	2·1108	6·17	0·340	31,500
I	2·4199	7·08	0·367	36,500
J	3·6250	10·60	0·411	43,100
K	2·0348	5·95	0·481	54,400
L	2·1725	6·35	0·550	66,100
M	2·0653	6·04	0·628	80,500
N	1·7823	5·21	0·730	100,000
O	2·1330	6·24	0·834	122,000
P	0·7442	2·18	0·982	155,000
Q	0·3314	0·99	1·029	166,000
	32·4323	94·87		

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BIOCHEMISTRY

Determination of the Biological Availability of Methionine and Cystine

There is no adequate method to determine the biological availability of the sulphur-containing amino-acids in the chick, but Schweigert and Guthneck[1] have described a technique for the rat. A method has therefore been developed for the bio-assay of methionine using White Leghorn chicks at 11–18 days of age: it can readily be modified to determine methionine plus cystine in terms of methionine equivalence. By difference an indication can be obtained of available cystine. The basal ration contains (per cent) 48·6 maize starch, 35 extracted soybean meal, 5 dried whey, 5 beef tallow, 6 mineral and vitamin mixture[2], 0·2 choline chloride and 0·2 glycine. All supplements to this ration replace part of the maize starch. Where test materials are calculated to contribute less 'metabolizable' energy than starch additional tallow is included to correct for the difference.

It was first established that methionine was the only amino-acid limiting growth and food utilization. This was accomplished by supplementing the diet with all other amino-acids singly and in various groups all in the presence of sufficient methionine to correct the known deficiency. In no case was any growth improvement observed, but it was shown that part of the methionine deficiency could be satisfied by L-cystine. A standard supplement of 0·4 per cent L-cystine was therefore included in all diets to eliminate the effect of any cystine in test proteins. When the methionine deficiency was corrected, chick growth was limited only by the protein level of the diet. A relatively low protein-level was chosen since this tends to maximize the response to methionine supplements when these are expressed as a proportion of the protein.

In the presence of excess L-cystine the growth response to additions of methionine is essentially linear on a logarithmic dose basis within the range of 0·01–0·1 per cent of the diet. Similarly, in the absence of added cystine the linear range is approximately 0·04–0·2 per cent (Fig. 1). These are the potential ranges for the bio-assay of methionine and methionine plus cystine respectively.

In order to satisfy the requirements for a bio-assay procedure it has been established that chick growth is a function only of the methionine or cystine content, that a constant growth response is obtained on repeated assay and that a linear and parallel response is obtained on the inclusion of graded amounts of standard and test materials in the diet. Using this technique, assays of biologically available methionine and cystine have been made on several

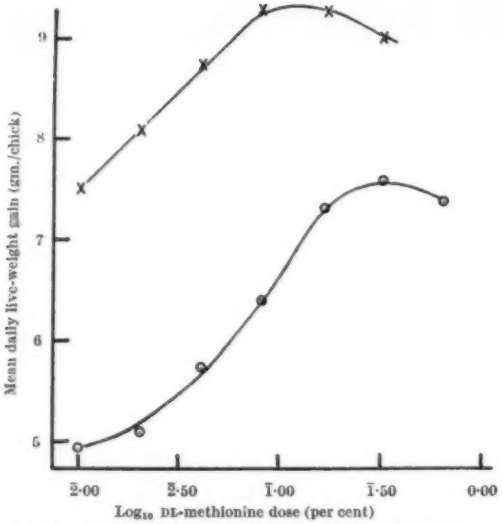

Fig. 1. Growth response of male White Leghorn chicks to graded supplements of DL-methionine. ○, DL-methionine; ×, DL-methionine plus 0·4 per cent L-cystine

soybean samples (Table 1). The second experiment quoted refers to three extracted soybean meals obtained from the samples examined for protein quality in animal feeding-stuffs by the Agricultural Research Council. The values for both available methionine and cystine are uniformly high in relation to chemical determinations. These samples had received varying heat treatments but none was shown to exhibit more than slight urease activity. On the other hand, a somewhat lower availability of these amino-acids was found in C-1 assay protein. This agrees with the observations of Turk *et al.*[3] and Ousterhout *et al.*[4] and also with our preliminary results, which suggest low availability of cystine in 'underheated' soybean meal samples (high urease activity).

In view of the observations of Fisher *et al.*[5] it was considered necessary to examine the possible effect of additional amino-acid inclusion on the response to standard supplements (Table 2). Neither supplements of L-glutamic acid nor a complete amino-acid mixture[5] significantly modified the growth pattern. This shows that under the conditions of this experiment alteration of amino-nitrogen or essential amino-acid status did not alter the response to methionine supplements.

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Table 1. Assays of Available Methionine and Methionine plus Cystine (based on Live-Weight Data)
In all cases cystine is expressed as methionine equivalence. Chemical determinations by a Moore and Stein technique. The fiducial limits are quoted at 5 per cent level of probability. All values expressed as gm./16 gm. nitrogen

	Methionine Assay	Methionine Fiducial limits	Methionine + cystine Assay	Methionine + cystine Fiducial limits	Cystine (by difference)
Experiment 1					
C-1 assay protein (soybean, 14·1% N)	0·97	1·22 0·74	1·68	2·14 1·36	0·71
Chemically determined	1·39		—		1·02
Availability (per cent)	70		—		69
Experiment 2					
Soybean 22 (8·01% N)	1·61	2·11 1·23	3·30	4·27 2·62	1·69
Soybean 23 (8·24% N)	1·74	2·30 1·33	3·16	4·08 2·51	1·42
Soybean 24 (7·81% N)	1·44	1·89 1·09	2·97	3·81 2·36	1·53
Mean of three samples	1·60		3·14		1·54
Chemically determined	1·78		—		1·80
Availability (per cent)	90		—		86

Table 2. Effects of L-Glutamic Acid and an Amino-Acid Mixture on the Response to Graded Supplements of DL-Methionine
Amino-acids added so that the total nitrogen is approximately equal to that of test proteins. Fiducial limits at 5 per cent probability

	Relative potency* per unit DL-methionine			
	In presence of 0·4 per cent L-cystine	Fiducial limits	In absence of cystine	Fiducial limits
Methionine	0·98	1·23	0·92	1·12
+ L-glutamic acid		0·78		0·75
Methionine + amino-acid mixture	0·92	1·16 0·74	1·01	1·23 0·83

* Computed according to Finney (ref. 6).

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Cation Toxicity and the Stability of Transition-Metal Complexes

Despite the many important contributions that have been made to the literature during the past century, no completely satisfactory theory of cation toxicity has been formulated. Blake[1] first concluded that there was a correlation between degree of toxicity and atomic weight (hence 'heavy-metal' poisons). Matthews[2] and Jones[3] later pointed out a relationship between metal ion toxicity and the 'solution pressure' (standard electrode potential) of the corresponding metals. Seifriz[4] considered various other chemical properties of the ions in his attempt to find a satisfactory chemical explanation for the relative toxicity of cations. Shaw[5-7], basing his arguments on results obtained with enzymes, postulated that metal ions killed aquatic organisms by combining with an essential group (probably —SH) on a key enzyme. Correlation with metal sulphide insolubility was demonstrated. An attempt was also made[8] to relate the relative inhibitory efficiency of bivalent metal ions toward the enzyme urease with metal complex stability. At that time (early 1953), however, the field of co-ordination chemistry had not reached a stage of sufficient maturity for such a comparison to be successfully made. The explosive growth that has occurred during the past decade in our knowledge of the chemistry of co-ordination compounds necessitates a re-examination of the intriguing possibility that there exists a relationship between metal complex stability and the toxicity of metal ions to living organisms.

In their classic paper, Irving and Williams[9] collected and critically examined available data on

Table 1. Toxicity of Transition-Metal Ions

Organism or enzyme	Ref.	Toxicity* of: Mn(II)	Fe(II)	Co(II)	Ni(II)	Cu(II)	Zn(II)
Daphnia magna	14	3·4	4·0	4·6	5·3	6·8	6·0
Urease	12	2·5	$4{\cdot}1_s$	$4{\cdot}2_s$	4·5	6·5	5·7
Stickleback	15	3·1	—	3·8	4·9	6·6	5·3
Guppy	7	2·2	—	3·3	4·7	6·5	5·7
Tadpole	7	2·1	—	4·0	4·1	6·5	5·0
Polycelis nigra	3	1·9	—	2·8	3·1	5·2	3·3
Diastase	16	0·79	—	2·0	3·0	3·9	1·8
Paramecium	17	1·0	—	1·2	$1{\cdot}2_s$	2·7	0·90
Fundulus eggs	2	0·30	1·0	1·1	1·2	4·2	0·60

* Toxicities are reported on a pI^* scale (refs. 5–7), that is, as the negative logarithm of the reported metal-ion concentration necessary to produce the particular level of biological response (LD_{50}, etc.) arbitrarily established by the researcher. pI values for enzymes have been discussed elsewhere (refs. 5 and 12).

the stability of complexes formed by bivalent ions of the first transition series. The Irving–Williams or 'natural' order: Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) was found to hold for the stability of nearly all complexes irrespective of the nature of the ligand. Theoretical justification for this important finding has been presented[8–10]. The relevance of the Irving–Williams order to biological processes in general has been eloquently emphasized by Williams[10,11]. Most of the examples used by him to illustrate his thesis were based on work on enzymes requiring metal ions and on metal-ion-catalysed reactions of model systems. Work on the applicability of the Irving–Williams order to the inhibition of enzymes by metal ions are not numerous, but recent work[12] has demonstrated that metal ions in the absence of phosphate buffer inhibit urease in the order: Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II). Since it seems reasonable to assume[6,7,12] that metal ions are toxic to living organisms because they combine with some ligand (probably itself part of an enzyme) necessary for life, one might naïvely expect the toxicity of transition-metal ions to follow a sequence similar to the Irving–Williams order. A more sophisticated view, however, would lead one to expect that complications involving competition of the host of ligands present in living systems for the metal ions might completely obscure such a simple relation[11]. This sophisticated view does not seem to be correct (Table 1); and the amazing fact is that all the data obtained throughout the years from the many different laboratories where work on the toxicity of metal ions to aquatic organisms has been done follows the toxicity sequence: Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II). The relationship holds also for urease, diastase, and other enzymes (Table 1). In media containing concentrated phosphate or other ligands with strong affinity for metal ions the sequence may, of course, be broken.

A comparison of toxicity with complex stability[11] is shown in Fig. 1.

The results of this investigation (Table 1, Fig. 1) establish the toxicity sequence proposed by me: Mn(II) < Fe(II) < Co(II) < Ni(II) < Cu(II) > Zn(II) as one that is of very general significance in aquatic biology and one that is also firmly based on the principles of co-ordination chemistry.

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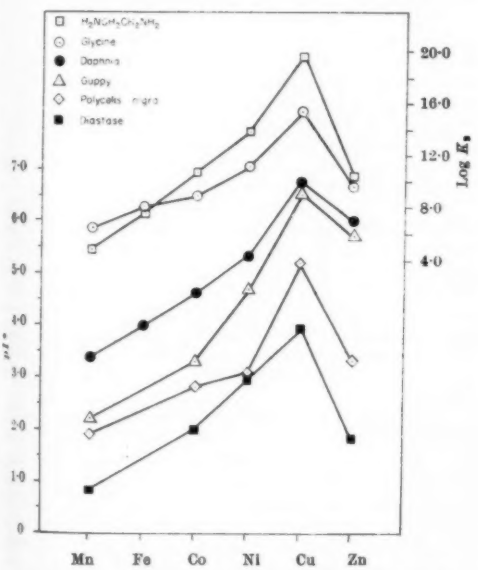

Fig. 1. Correlation of transition-metal complex stability (ref. 11) (two upper curves, right ordinate) with transition-metal ion toxicity (lower curves, left ordinate). Toxicities in pI^* units were obtained from Table 1

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A Simplified Culture Medium for the Production of Collagenase

Present methods for the production of *Clostridium histolyticum* collagenase depend chiefly on growth of the organism in complex culture media[1,2]. It is the purpose of this communication to describe a relatively simple, partially defined medium for the production of large quantities of highly active fermentation broths of *Cl. histolyticum* collagenase. The medium described appeared to facilitate the isolation and purification of collagenases which were essentially free of non-specific proteinases[3].

The organism used in these experiments was *Cl. histolyticum* 47*Q*–5 obtained from the Army Medical Service Graduate School, Washington, D.C. A metabolically constant source of collagenase was prepared by pre-treating the organism in the following manner: A 24-hr. culture was injected intragluteally into Swiss mice through five serial passages. Isolation (muscle exudate) was accomplished and growth continued in a seed medium of composition: casein hydrolysate, enzymatic (General Biochemicals, Chagrin Falls, Ohio), 16 gm.; sodium succinate. 5 gm.; sodium pantothenate, 0·1 mgm.; pyridox-

amine, 0·2 mgm.; nicotinamide, 0·3 mgm.; uracil, 1·5 mgm.; riboflavin, 10 μgm.; biotin, 0·1 μgm.; vitamin B_{12}, 63·0 μgm.; and distilled water to 1,000 ml. (*p*H 7·6). Stock cultures of *Cl. histolyticum* were maintained in 'Pyrex' tubes (25 × 200 mm.) containing 40 ml. of this medium to which 1 gm. of dry, fat-free bovine heart was added. The stock cultures were transferred at monthly intervals and were refrigerated in the interim. The culture medium used in the production of collagenase consisted of the following constituents in 1 l. of medium: Solution 1, casein hydrolysate (enzymatic), 40 gm.; sodium succinate, 1·25 gm.; sodium thioglycollate, 1·0 gm.; $Na_2HPO_4.12H_2O$, 5·8 gm.; KH_2PO_4, 0·48 gm.; gelatin, 2·0 gm. Solution 2 (stock vitamin preparation), calcium pantothenate, 1·0 mgm.; pyridoxamine, 2·0 mgm.; riboflavin, 0·1 mgm.; nicotinamide, 8·0 mgm.; uracil, 15·0 mgm.; biotin, 10·0 μgm.; vitamin B_{12}, 63·0 μgm. Solution 3, dextrose (50 per cent), 5·0 ml. After preparation of solution 1, the incomplete medium was adjusted to *p*H 7·6 with 5 *N* sodium hydroxide. Solution 2 was then added and the combined medium was then placed in an autoclave for 15 min. at 15 lb./sq. in. After cooling, Seitz-filtered glucose (solution 3) was added aseptically. 9-l. 'Pyrex' carboys containing 6·5 l. of medium were used. The inoculum was prepared in 500 ml. of broth of the same composition as that being used to produce collagenase. The maximum yield of enzyme was obtained after 48 hr. incubation at 24–25° C. The bacterial cells were removed by filtration through 'Hyflo Super Cel' and the *p*H of the filtered broth adjusted to 7·5–7·6 with 5 *N* sodium hydroxide. Collagenase activity was measured by hydrolysis of undenatured Achilles tendon collagen as described in ref. 3. One collagenase unit was defined as the amount of activity catalysing the solubilization of 1 mgm. of 40–60 mesh collagen under standard conditions. It was found that the foregoing relatively simple procedure provided broths with collagenase activity that averaged 107 units of enzyme a ml. The average yield of purified preparations of collagenase expressed as collagenase *A* was 140 mgm./l. of fermentation beer.

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Crystallization of Deuterated Ribonuclease

We have prepared deuterated bovine pancreatic ribonuclease (Armour, lot 381–059) by the procedures of Hermans and Scheraga[1]. Both $RNaseH_{20}$ (all but 20 exchangeable H's exchanged) and RNase all-deuterated crystallized from 55 per cent (v/v) aqueous 2-methyl-2,4-pentanediol (deuterated solvent) in form II[2]. The intensities of the X-ray reflexions along the principal axes were identical within experimental precision to those of non-deuterated crystals out to $2\theta = 60°$ (1·5 Å. resolution); at higher angles RNase gives poor reflexions. The lattice constants were identical to those of undeuterated crystals within the normal variation (± 0·05 Å. and ± 0·03°). The small differences between deuterium bonds and hydrogen bonds do not change the structure of RNase within the limits observable by X-ray diffraction. Detectable changes might be produced in the case of a crystallizable protein with a transition temperature near room temperature instead of the high transition temperature (61° C.) of RNase[3].

The deuterated crystals were prepared by Miss Sylvia Scapa and Mrs. H. R. Bello; diffraction patterns were taken by Miss E. De Jarnette.

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Properties of Xanthine Dehydrogenase in the Silkworm, *Bombyx mori* L.

The enzyme which catalyses the oxidation of purine derivatives xanthine and hypoxanthine to uric acid has been demonstrated in several insects[1–5]. In the silkworm, *Bombyx mori*, it functions as a dehydrogenase. This inference is based on the fact that the formation of uric acid and oxygen consumption of the fat body homogenates show marked increases only when methylene blue is present as a hydrogen carrier[6,7]. The inference seems to be justifiable from the work of Remy *et al.*[8] using chicken liver. To gain further knowledge of the nature of xanthine dehydrogenase in the silkworm, it will be desirable to use the highly purified enzyme as in milk[9,10] and liver[11] oxidase preparations.

The present report deals with preliminary work on the properties of the xanthine dehydrogenase in the silkworm. The enzyme was prepared from the fat body according to the methods of Kielley with a few modifications (Hayashi, unpublished). The enzyme activity was determined from oxygen consumption and uric acid formation by a method similar to that described in the previous paper using crude tissue homogenates of silkworm larvæ, with methylene blue as a hydrogen carrier between xanthine dehydrogenase and molecular oxygen[7].

Purified preparations of the silkworm enzyme are yellowish-brown in colour. The enzyme has two absorption bands with maxima at 360 and 405–410 mμ, the latter disappearing when reduced by hydrosulphite. The absorption spectrum of the silkworm enzyme is similar to that of the chicken liver enzyme[8] and aldehyde oxidase[12]. The difference spectrum was not conspicuously characteristic of flavin and is probably a compound of flavin plus an unknown group.

In recent work it has been reported that xanthine oxidative enzyme from some animal tissues contains iron, molybdenum and flavin as the prosthetic groups. The mammalian liver xanthine oxidase of Kielley[11] has an Fe : Mo : flavin ratio of 4 : 1 : 1 and the chicken liver xanthine dehydrogenase of Remy *et al.*[8] has a ratio of 8 : 1 : 1. From the purified preparation of xanthine dehydrogenase from silkworm fat body, it was determined that the ratio is 9 : 1 : 1, thereby showing closer resemblance to the latter rather than the former prosthetic group.

Milk[10] and chicken liver[8] enzyme are capable of oxidizing a number of oxypurines and aldehydes as well as reduced diphosphopyridine nucleotide (DPNH), but slowly with pterines. The silkworm xanthine dehydrogenase oxidized xanthine, hypoxanthine, guanine, adenine, aldehydes, 2-amino-4-hydroxypteridine and xanthopterin, while the oxidation of DPNH by the enzyme proceeded faster only when cytochrome *c* was added as an electron acceptor. In these substrates 2-amino-4-hydroxypteridine and xanthopterin were oxidized faster than the purines such as hypoxanthine and xanthine.

A wide variety of electron acceptors has been used in the enzyme system. Methylene blue, tetrazolium salts, indophenol, diphosphopyridine nucleotide (DPN), cytochrome *c*, flavin adenine dinucleotide (FAD), and sodium pyruvate have been shown to be acceptors. The enzyme activity of the reaction systems mentioned showed its maximum rate with methylene blue; DPN and FAD showed the same rate.

The activity of the silkworm enzyme has a wide *p*H range of 6–11 with a maximum velocity at *p*H 8·4. A number of substances have been found to be possible inhibitors of silkworm enzyme. Among them the more effective inhibitors are potassium cyanide and *o*-iodobenzoic acid, whereas xanthine oxidase inhibitors such as ascorbic acid, *p*-aminophenol, hydroxylamine, quinine, and antimycin *A* were not recognized as inhibitors.

As shown from the results stated above, the property of the xanthine enzyme system in silkworm fat body is not similar to that of mammalian liver enzyme but bears a striking likeness to chicken liver enzyme except in the case of substrate specificity. From this, it is inferred that the properties of xanthine oxidative enzymes vary from source to source.

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Conversion of 2-^{14}C-Pteroyl-L-Monoglutamic Acid into Isoxanthopterin in *Drosophila melanogaster*

2-^{14}C-Pteroyl-L-monoglutamic acid (0·5 mc. per millimole, 1 mgm.) was incorporated into banana food medium (100 gm.), and adult flies of *Drosophila melanogaster* (Oregon, wild-type) were allowed to feed and lay eggs on this medium. Larvæ (six days old) were removed from this medium, transferred to moist filter paper and allowed to pupate. Pupæ (100; 86 mgm.; not less than 48 hr., not more than 56 hr. pupation) were crushed with sand and 0·5 *N* aqueous ammonia solution (0·5 ml.) and heated on a steam-bath (10 min.). Solids were centrifuged off and supernatant liquid spotted on Whatman 3 *MM* paper. The solids were washed again with 0·5 *N* aqueous ammonia solution (0·4 ml.), centrifuged off and supernatant liquid added to the first spot. The paper area was cut out and radioactivity measured by immersion in a solution of 0·3 per cent diphenyloxazole in toluene (2 ml.) and counting with a 'Panax' scintillation counter (background, 1·096 counts/sec.; background + sample, 1·357 counts/sec.; sample, 0·26 count/sec.; standard error, ± 0·035 count/sec.). The radioactive area of the paper was chromatographed on 3 *MM* paper with propanol/5 per cent aqueous acetic acid (2 : 1 vol. by vol.) as developing solvent. To improve spot resolution, the paper was developed three times. On viewing in 365-mμ light, a bright purple fluorescent area was seen. This was cut out and chromatographed on 3 *MM* paper with butanol/acetic acid/water (4 : 1 : 5 vol. by vol.) (triple development). After air-drying, the paper was viewed in 365 mμ light and the fluorescent spots of isoxanthopterin (purple) and 2-amino-4-hydroxypteridine-6-carboxylic acid (bright blue) cut out. The acid spot was measured for radioactivity (background, 0·65 count/sec.; background + sample, 1·10 counts/sec.; sample, 0·45 count/sec.; standard error, 0·033 count/sec.). The isoxanthopterin spot was further purified by chromatography on 3 *MM* paper with *tert.*-butanol/pyridine/water (50 : 15 : 35 vol. by vol.). The isoxanthopterin spot was measured for radioactivity (background, 0·65 count/sec.; background + sample, 0·76 count/sec.; sample, 0·11 count/sec.: standard error, 0·030 count/sec.).

The counting efficiencies of the isoxanthopterin spot and the original paper area (after development) were measured by adding known weights of 2-^{14}C-pteroyl-L-monoglutamic acid and determining the radioactivity of the paper areas. The counting efficiency of the isoxanthopterin spot was about 12·1 per cent and that of the original paper area about 6·7 per cent. The isoxanthopterin therefore represented about 20 per cent of the radioactivity present in the sample, the remainder being accounted for by the 2-amino-4-hydroxypteridine-6-carboxylic acid.

The pterin isoxanthopterin is widely distributed in Nature. It has been suggested that it is the end-product of the catabolism of folic acid (pteroyl-L-monoglutamic acid), being formed by the following steps[1,2]:

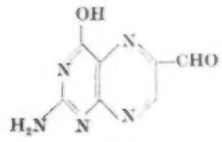
pteroyl-L-monoglutamic acid $\xrightarrow[(2)\ \text{hydrolysis}]{(1)\ \text{dehydrogenation}}$ 2-amino-4-hydroxypteridine-6-aldehyde $\xrightarrow[\text{oxidase}]{\text{xanthine}}$ 2-amino-4-hydroxypteridine-6-carboxylic acid $\xrightarrow{\text{decarboxylation}}$ 2-amino-4-hydroxypteridine $\xrightarrow[\text{oxidase}]{\text{xanthine}}$ isoxanthopterin

Isoxanthopterin can be readily isolated from the pupæ of *D. melanogaster*, and it has been shown that 6,7-^{14}C-2-amino-4-hydroxypteridine is converted only into radioactive isoxanthopterin in pupæ[3]. Work with mutants of *D. melanogaster*, which do not produce isoxanthopterin but instead accumulate large amounts of 2-amino-4-hydroxypteridine and biopterin, shows that this failure to produce isoxanthopterin is due to deficiency of the enzyme xanthine oxidase[3].

D. melanogaster larvæ and pupæ can synthesize folic acid in limited amount but require to be supplied with folic acid for their normal growth[4].

The experimental results establish that some of this supplied folic acid is converted into isoxanthopterin by the pupæ.

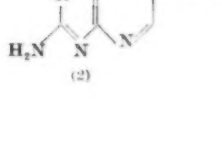
D. melanogaster mutants that do not form isoxanthopterin accumulate 2-amino-4-hydroxypteridine and biopterin. The latter is not formed from the former[3]. If the biogenetic scheme suggested is correct, then if xanthine oxidase is lacking, large amounts of 2-amino-4-hydroxypteridine-6-aldehyde should be formed. This could be converted to biopterin by the following steps:



(1)

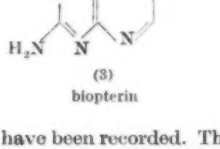
2-amino-4-hydroxypteridine-6-aldehyde

$CH_3COCOOH$ (or biochemical equivalent) and enzyme(s) →



(2)

reduction →



(3)

biopterin

Analogies for the first step have been recorded. The enzymatic condensation of pyruvic acid with acetaldehyde and propionaldehyde yields acetoin and methyl acetoin[5]. Ultra-violet irradiation of pyruvic acid and benzaldehyde gives benzacetoin[6]. Benzacetoin is formed from benzaldehyde in alcoholic fermentation[7].

In accord with this prediction, mutants of *D. melanogaster* lacking xanthine oxidase accumulate biopterin as well as 2-amino-4-hydroxypteridine.

I am grateful to Prof. F. Weygand for a gift of 2-^{14}C-pteroyl-L-monoglutamic acid, to Dr. A. H. Ward for assistance with the radioactivity measurements and to Dr. M. Hollingsworth for the *Drosophila* stock.

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PHYSIOLOGY

Soluble Collagen in Bone

CONSIDERABLE efforts have been made, so far, to investigate the physico-chemical as well as the biochemical nature of soft tissue collagen such as skin and tendon, especially. In refs. 1–3 it is suggested that the soluble collagen is the precursor of collagen. However, only scanty information has been provided in regard to the important role of bone collagen in the formation of bone[4]. Trials in refs. 5 and 6 have not been successful in proving the existence of soluble collagen.

To clarify the mechanism of ossification, we carried out experiments on the extraction and refinement of bone collagen. The first problem of the extraction of bone collagen is to eliminate the bone mineral which occupies the major part of bone. Some attempts have been made to solve this problem and de-calcification by dilute acids has been found to be most suitable. We took special care to prevent a loss of soluble collagen, since it might be missed in the process of demineralization; this enabled us to prove that the soluble collagen was obtainable also from bone.

Cortical bone of fresh bovine femur was pulverized with dry ice to obtain 100–200 mesh powder after complete removal of attached soft tissues and defatting with ether, then freed from remaining soft tissues by flotation[7]. This sample was stored at − 20° C. As for de-calcification, bone powder thus prepared was suspended in 0·1 *N* hydrochloric acid and dialysed at 4° C. in cellulose tubing, average pore radius 24 Å. (Visking Co.), against a flow of 0·1 *N* hydro-

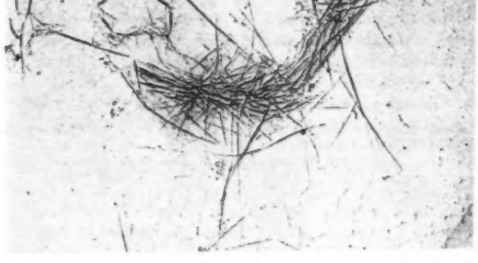

Fig. 1. Reconstituted fibres obtained from the soluble fraction of cortical bone (bovine femur) show a needle-like shape under the microscope. (× 300)

Fig. 2. Electron-micrograph of the reconstituted fibrils (*p*H 5·5, McIlvaine buffer) obtained from the soluble fraction of cortical bone (bovine femur). This shows the characteristic periods of 640 Å.; fixed with phosphotungstic acid, shadowed with palladium–platinum

chloric acid (10 l./day). After completion of demineralization (about 2 weeks), the non-dialysable portion of the extract in the tubes was separated from the residue by centrifugation (3,600*g* for 10 min.), sealed in the tubes and dialysed again at 4° C. by renewing the distilled water (10 l./day for 4 days).

Precipitate which appeared during dialysis was dissolved in McIlvaine buffer (7·1 vol. of 0·2 *M* sodium phosphate aq.: 12·9 vol. of 0·1 *M* citric acid aq., *p*H 3·8) after centrifugation (3,600*g* for 10 min.) and the supernatant was dialysed at 4° C. against McIlvaine buffer (11·6 vol. of 0·2 *M* sodium phosphate aq.: 8·4 vol. of 0·1 *M* citric acid aq., *p*H 5·5–5·6) containing 2 per cent sodium chloride followed by concentration with 50 per cent gum arabic.

A crystal of sodium chloride was added occasionally to promote the formation of fibre. As shown in Fig. 1, fibres showed a needle-shape under the microscope. They were rinsed with water repeatedly before fixation with 1 per cent phosphotungstate, washed again and suspended in water, then placed on grids and desiccated with calcium chloride. Thus, the electron-microscopical observation revealed the characteristic pattern of collagen with a 640-Å. periodicity. This collagen fibre subjected to a repetition of this process gave the typical pattern of reconstituted collagen fibre with 640-Å. periodicity (Fig. 2).

In addition, the amino-acid content of this reconstituted collagen was similar to that of so-called bone collagen, and it seems probable that the collagen fibre described is identical with collagen also from its contents of proline and glycine.

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Flow of Blood in the Forearm of Persons acclimatized to Heat

It is known, from experiments performed in climatic rooms in temperate countries, that the flow of blood in the forearm increases when people are exposed to heat, and that the magnitude of this increase diminishes progressively if the exposure to heat is repeated on successive days[1,2]. At the end of these experiments, however, the flow of blood in the forearm was always higher during the exposure to heat than it was before the experimental subjects were first exposed to heat. It cannot, therefore, be decided from these observations whether the flow of blood in the forearms of people who are fully acclimatized to heat, by long residence in a hot environment, are higher or lower than those of people who are not acclimatized to heat. An investigation into the flow of blood in the forearms of indigenous peoples of a hot, humid climate would provide information on the effects of long-term acclimatization to heat, but no measurements of blood-flow in tropical subjects appear to have been made until now.

In order to supply these data, the flow of blood in the forearms has been measured, by venous occlusion plethysmography in twenty-one male Asian subjects living in Singapore. The subjects were fifteen Chinese, four Indians and two Malays; their average age was 23·1 yr. (*S.D.* = ±3·8) and their mean body-weight was 57·7 kgm. (*S.D.* = ±7·9). All subjects had been born in Singapore, and they were therefore 'naturally' acclimatized to heat[3]. They were tested at the environmental temperatures of Singapore (mean dry-bulb temperature, 28·3 ± 1·1° C.; mean wet-bulb temperature, 25·2 ± 0·7° C.) and they wore their normal clothing[4]. Each subject lay on his back for 90 min. before any measurements were made. Blood-flows were measured in four sets of five flows; each set lasted 2·5 min. and was separated from the next set of readings by a time interval of 15 min. The mean blood-flow for any one subject therefore represented the average of twenty flows taken over a period of approximately 1 hr.

The mean blood-flow in the forearms of the twenty-one subjects was 2·70 ml./100 ml. forearm/min., with a standard deviation of ±0·88 ml. There was some evidence that the flows of blood in the forearms of these subjects were not greatly altered by enclosure of the arm in a water-filled plethysmograph. Thus, during the hour in which blood-flows were measured, there were only random variations in the flow readings. Progressive cooling or warming of the forearm might have been expected to result in trends in the blood-flows over a period of 1 hr. The temperature of the water in the plethysmograph was 35·0° C.; the skin temperature of the forearm when it was in the plethysmograph must have been close to 35° C. In a similar group of subjects to that taking part in the present investigation, at a room temperature of 29·6° C., the mean skin temperature of the forearm when it was exposed to air was 34·88° C. (ref. 4). The skin temperature of the forearm while measurements of blood-flow were being made could not therefore have been very different from that before the arm was inserted into the plethysmograph.

From these considerations it seems reasonable to assume that the figure of 2·70 ml./100 ml./min. may be accepted as an estimate of the rate of blood-flow through the forearms of young men, at rest, in a hot, humid climate. This figure is of the same order of magnitude as flow-rates of 2·6–3·1 ml./100 ml./min., which have been reported for the clothed forearms of people at normal room temperatures (18·5°–20·0° C.) in England[5] and in North America[6]. The forearms of young men in Singapore are habitually bare; the skin temperatures of their forearms are, however, higher than those of the clothed forearms of people in England and North America[4]. For this reason, it might be expected that the flows of blood in the forearms of the subjects in Singapore would be greater than those of people in temperate countries. The experiments reported here have not provided any evidence to support this. It is possible that the relatively low values for blood-flow in the forearms of the Singapore subjects may be related to the fact that people acclimatized to heat in Singapore have low mean arterial blood pressures[7].

It is important to stress that these conclusions apply only to men who are at rest and who are not visibly sweating. Increased activity or the onset of

sweating might result in a greatly increased blood-flow through the forearms of these subjects but evidence for this is, at present, lacking.

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New Indirect Fick Procedure for the Determination of Cardiac Output

The indirect Fick method for determining cardiac output, which requires neither catheterization nor arterial puncture, would be the method of choice but for one serious drawback, namely, the difficulty of obtaining a reliable value for the carbon dioxide tension in (oxygenated) venous blood. It is now generally recognized that, when the breath is held, no equilibrium between alveolar and mixed venous P_{CO_2} is attained in the 15–20 sec. before recirculation intervenes. It has been proposed to plot the increase in pressure of alveolar carbon dioxide, P_{CO_2}, while the breath is held, and to compute the mean value of venous P_{CO_2} by means of extrapolation[1]. Since alveolar P_{CO_2} cannot be measured while the breath is held, this method requires the determination of alveolar P_{CO_2} at the end of a number of successive periods of holding the breath of different lengths. This results in considerable scatter of the experimental points and, thus, in an appreciable degree of uncertainty regarding the value for alveolar P_{CO_2}.

It was shown earlier[2] that, in the case of re-breathing in a bag initially filled with pure oxygen or with an oxygen – carbon dioxide mixture, the alveolar carbon dioxide tension after the nth breathing cycle, p_n, is given by an inhomogeneous linear second-order difference equation with constant coefficients:

$$p_n = a + bp_{n-1} + cp_{n-2}$$

and that the second exponential, which is a function of the tidal volume/dead space ratio, becomes negligible after the first or second breathing cycle. From then on, the process is a simple exponential one. This means that, after the first few breaths, the p_n vs. p_{n-1} plot is a straight line:

$$p_n = a + bp_{n-1}$$

or, if p_1 be the initial value of p:

$$p_n = a(1 + b + b^2 - - - - - b^{n-2}) + b^{n-1}\,p_1$$

The sum of the series in brackets being $(b^{n-1}-1)/(b-1)$, we have:

$$p_n = \frac{ab^{n-1} - 1}{b - 1} + b^{n-1}p_1$$

For $n \to \infty$, a positive finite end value (p_∞) will be approached if $0 < b < 1$.

This value is given by:

$$p_\infty = a\,\frac{0 - 1}{b - 1} = \frac{a}{1 - b}$$

Whether the asymptotic value is higher or lower than the initial value depends on the numerical values of a, b, and p_1.

For p_2 to be higher than p_1 (also $p_3 > p_2$, etc.) we have:

$$p_2 > p_1 \to a + bp_1 > p_1$$

or, since $bp_1 + (1-b)p_1 = p_1$, the condition can be written as:

$$a > p_1\,(1 - b)$$

Similarly, $p_n < p_{n-1}$ if $a < p_1\,(1-b)$.

The first case corresponds to that in which the initial partial pressure of carbon dioxide in the bag is less than the true mean value of venous P_{CO_2}, and the second to that in which it exceeds this value. The procedure is extremely simple. At the end of a normal expiration, the subject is connected, by means of a three-way tap, to a 1-litre bag filled with an oxygen-rich oxygen–carbon dioxide mixture and re-breathes for a period not exceeding 15–16 sec. At the end of each expiration, the concentration of carbon dioxide (per cent) in the bag is measured with the aid of a fast carbon dioxide analyser. Scatter is minimized by having the subject re-breathe at a fixed tidal volume and at a fixed frequency (checked by metronome).

The p_n versus p_{n-1} plot is drawn, and if, after the first few points, a straight line is obtained, its slope and intercept of y (that is, b and a, respectively) are determined by the method of least squares, and p_∞ is calculated.

Under certain conditions, such as unequal ventilation, etc., no straight line is obtained. In those cases the method is not applicable. The procedure is illustrated by Figs. 1, 2 and 3. Fig. 1 shows the record obtained with an infra-red analyser; the percentages of carbon dioxide after successive breathing cycles are plotted against time in Fig. 2; the corresponding p_n versus p_{n-1} plot is shown in Fig. 3. From the values for a and b (0·856 and 1·071 (760 − 47/100) = 7·636, respectively), p is found to be 52·30 mm. mercury.

Values obtained with this method possess a high degree of reproducibility, as has been found in our laboratory and at the Lovelace Foundation. Cardiac

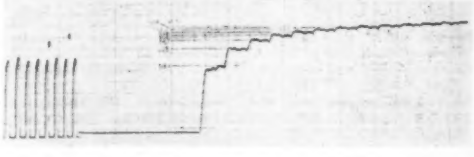

Fig. 1. Record obtained from an infra-red analyser. Concentration of carbon dioxide in bag at the end of successive expirations

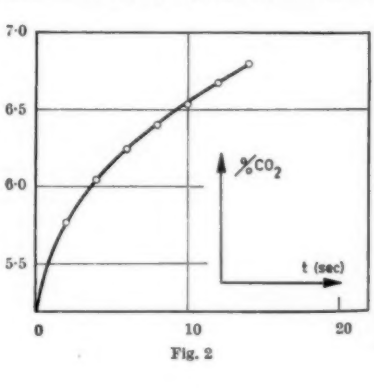

Fig. 2

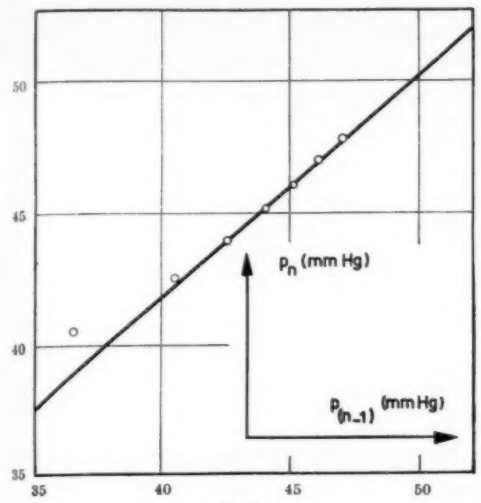

Fig. 3

output values of resting normal subjects lie, as a rule, between 5 and 6 litres/min. At the time of writing, Dr. Mostyn (University of Alberta, Canada) is engaged in a series of experiments where the values for cardiac output obtained by the method described are compared with those found by the direct Fick method.

A full report, including discussion of errors, will be published shortly in the *Acta physiol. et pharmacol. neerlandica*.

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Œstrus-inducing Activity of Testosterone in the Ewe

On the basis of results obtained with testosterone administered, together with pregnant mare serum gonadotrophin, to anœstrous ewes Cole[1] suggested that androgen may be responsible for the induction of œstrous behaviour in the sheep. Robinson[2], however, has shown that œstrogen given following a period of progesterone priming will induce both behavioural and vaginal œstrus in spayed ewes, and it is probable that normal cyclic œstrous behaviour is due to an interaction of these two hormones. In the course of work on the interaction of several steroid hormones on behavioural and vaginal œstrus in spayed ewes, a preliminary trial showed that a single dose of testosterone or of testosterone propionate resulted in mating behaviour in a number of animals. Consequently, a factorial design test, using 72 spayed ewes, was conducted to examine this phenomenon and to define the nature of the apparent œstrous response. The variables introduced (with $n = 12$) were priming treatment (no progesterone or 10 mgm. per day for 12 days)[3], and dose of androgen (1·0, 3·2 or 10·0 mgm.). The androgen was testosterone propionate. Sexual receptivity of the ewes was determined using 8 intact rams fitted with 'Sire-sine' harnesses and crayons[4] and the vaginal response was determined by smears taken for four days following injection of androgen[5]. Results are presented in Table 1.

Table 1. Behavioural and Vaginal Response to Testosterone Propionate administered to 72 Ewes with or without Progesterone Pretreatment

	Dose of testosterone propionate (mgm.)	Treated with progesterone	Not treated with progesterone	Total
Behavioural response ($n = 12$)	1·0	6	4	10
	3·2	10	11	21
	10·0	12	12	24
	Total	28	27	55
Vaginal response ($n = 12$)	1·0	1	—	1
	3·2	1	1	2
	10·0	—	—	—
	Total	2	1	3

Full sexual receptivity was induced by testosterone propionate and the response appeared unaffected by previous administration of progesterone, and so differed markedly from that to œstrogen[3]. The dose response effect ($P < 0{\cdot}001$) had the following form:

$$y = 42{\cdot}8\,x + 41{\cdot}6$$

where $\sin^2 y$ = proportion of ewes responding, and $x = \log_{10}$ dose androgen, expressed as free testosterone (mgm.). The median effective dose was 1·20 mgm., which suggests an œstrus-inducing activity comparable to that of œstriol[6].

Vaginal smears from only 3 ewes showed evidence of epithelial cornification and desquamation characteristic of œstrogen activity. Only one of these ewes demonstrated behavioural œstrus, suggesting that these apparent responses were artefacts. On the day of service, vaginal smears consisted of thin watery mucus with leucocytes and spermatozoa. On the 3 succeeding days there was massive leucocytic invasion, and many smears consisted entirely of thin mucus and leucocytes with some cellular debris. This is in marked contrast to the picture when behavioural œstrus is induced with œstrogen, where smears 2 and 3 days after service consist almost entirely of desquamated cornified cells, and leucocytes are almost absent[3,5].

The effect of testosterone in inducing behavioural œstrus in this test appears to have been one direct on the central nervous system. It does not appear to have been a precursor for œstrogen, in which case the vaginal smears would have presented evidence of œstrogen activity. It is well established that libido in women is due largely to androgen, presumably of adrenal origin[7], and the presence and production of androgens in ovarian tissue have been demonstrated[8]. Cole, Hart and Miller[1] observed that some ewes treated with pregnant mare serum gonadotrophin and 100 mgm. of testosterone exhibited homosexual activity. The median effective dose of testosterone, as assessed from our test, is only about 1 per cent of the dose administered by these workers. No abnormal behaviour was observed at our dose-rates.

Œstrus has two distinct components—anatomical and psychic—and these can now be differentiated in the ewe. Œstrogen, alone, commonly will induce vaginal œstrus without behavioural œstrus[3,5]. Œstrogen, following a period of progesterone influence, will induce both vaginal and behavioural œstrus[2,3]. Testosterone will induce behavioural œstrus minus vaginal œstrus.

Three conclusions may be drawn:

(1) Œstrous behaviour may be attributed to direct action on the central nervous system of an appropriate hormone, and not to the conditioning of peripheral receptors in the uterus[2] or in the vagina.

(2) The pattern of the behavioural response to gonadal hormone is determined by the sex of the recipient rather than by the nature of the hormone administered.

(3) It is not suggested that androgens normally are responsible for cyclic œstrous behaviour in the sheep. It is well established that œstrogen, in association with progesterone, has that role. It is clear, however, that the terms 'œstrogenic' and 'androgenic', in their commonly accepted context, are largely meaningless when applied to behavioural responses.

This project forms part of a programme on the physiology of reproduction in the sheep financed by the Australian Sheep and Wool Research Committee. Testosterone propionate was kindly donated by G. D. Searle and Co., Chicago, and progesterone by Fawns and McAllan Pty., Ltd., Melbourne.

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IMMUNOLOGY

Transfer of Immediate (Anaphylactic) Hypersensitivity by Homologous Bone Marrow or Spleen Cells

It has been repeatedly shown that lymph node cells from immunized donors transferred to irradiated recipients can transfer a specific antibody production and immunity state[1-3]. We postulated that by implanting cells from a hyper-sensitized donor into a recipient made tolerant by whole-body irradiation, it would be possible to transfer the immediate type of hypersensitive state. The present experiments demonstrate that the anaphylactic state can be obtained by transplantation of homologous bone marrow or spleen cells from sensitized donor guinea pigs into whole-body irradiated homologous recipients.

Guinea pigs of a heterozygous randomly bred colony were used. They were between 300 and 400 gm. body-weight. Donor animals were injected beneath the skin with 2 × 0·05 ml. of horse serum emulsified in Freund's adjuvant mixture. Six days later, bone marrow cells obtained from the femurs, tibiæ and humeri were suspended in cold Tyrode's solution. The suspension of spleen cells was prepared by squeezing the mashed tissue through gauze; finally, cell suspensions were filtered through a loose cotton pellet. In a group of sensitized animals injected intravenously six days later with 0·15 ml. of horse serum no signs of anaphylactic reaction were observed (four animals). Animals challenged on the ninth day (two animals) manifested moderate symptoms, and on the fifteenth day three tested animals exhibited fatal anaphylactic reaction.

In several experiments, previously irradiated animals and twenty-two unirradiated ones received cell suspensions intravenously. Irradiated animals were exposed to 300, 400 or 700 r. whole-body X-irradiation under the following conditions: 220 kV., 15 m.amp., 0·5 mm. of copper and 1 mm. of aluminium, 40 cm. from target to animal skin, 83 r./min. According to the cells which they received the recipients were divided into three sub-groups. The first obtained a mixture of about 11 × 10^7 viable nucleated bone marrow cells and 10 × 10^7 viable spleen cells. The second sub-group received 11 × 10^7 bone marrow cells only and the third 10 × 10^7 spleen cells only. Viability was estimated by the number of stained (dead) cells in a 0·2 per cent solution of eosin in Tyrode's solution. Cell suspensions were injected into the jugular vein within 3–5 hr. after irradiation under light local anæsthesia (2 per cent novocain).

Nine days and in some cases 16 days after the cell transfer animals were challenged by intravenous injection of 0·15 ml. horse serum. (Not all the irradiated animals had survived up to that time.) The ratio of the number of reacted to the number of tested animals as well as anaphylactic index for all experimental groups are shown in Table 1.

As seen in Table 1, all the irradiated animals showed anaphylactic responses (in a few minutes after challenge). The degree and duration of the reaction were not the same in all animals but always pronounced; three animals died of shock. Only some of the unirradiated animals showed very weak symptoms which could be attributed to anaphylactic response.

From these results it seems possible to conclude that:

(1) By injection of bone marrow or spleen cells it is possible to transfer the immediate type of hypersensitivity into recipient animals. The intensity of the

Table 1

Recipient animals group	Day of challenge (post-transfer)	Bone marrow + spleen cells		Bone marrow cells		Spleen cells		Total	
		Reacted to test	Anaphylactic index*	Reacted to test	Anaphylactic index*	Reacted to test	Anaphylactic index*	Reacted to test	Anaphylactic index*
Unirradiated	9	0/3	0	2/12	0·1	2/5	0·7	4/20	0·2
	16	0/2	0	—	—	—	—	0/2	0
300r.	9	—	—	8/8	2·0	3/3	2·0	11/11	2·0
400r.	9	6/6	2·8	4/4	3·0	6/6	3·5	16/16	3·1
	16	4/4	3·8	—	—	—	—	4/4	3·8
700r.	9	4/4	3·8	5/5	2·4	2/2	2·0	11/11	2·7

* Anaphylactic index expresses the relative degree of anaphylactic state. It is calculated by multiplying the number of animals showing fatal symptoms by 5, severe symptoms by 4, moderate symptoms by 3, mild symptoms by 2, very weak or uncertain symptoms by 1, without symptoms by 0, and dividing the sum of the products by the total number of animals challenged. This is a slightly different system from that described in ref. 5.

anaphylactic response showed that the ability of spleen and bone marrow cells to induce anaphylactic response by transfer was about the same.

(2) Passive transfer of antibodies as a reason for reaction is not probable because (*a*) sensitized animals challenged on the sixth day did not show anaphylactic symptoms; (*b*) in this case unirradiated animals should have reacted also. This second reason seems to exclude direct antigenic stimuli of recipient animals if antigen were transferred together with cells from sensitized donor guinea pigs.

(3) The success of transfer seems to depend mainly on the possibility that the recipient animal rejects transferred cells. Failure to induce anaphylactic reaction is probably due to rejection of the sensitized cells before they can produce enough anti-horse serum antibodies. If the transferred cells have a better opportunity to colonize the recipient tissues and mature some days in them, the production of antibodies may be sufficient and an injection of horse serum can elicit an anaphylactic reaction. In this experiment the necessary conditions for successful cell transfer were obtained by irradiation.

(4) The number of cells necessary to achieve the anaphylactic state in irradiated recipient animals was evidently much smaller than in the case of normal recipient animals[4].

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Agarophoresis and Immuno-electrophoresis of the Proteins of Gastric Juice

A number of authors have used paper and freely moving boundary electrophoresis for investigating proteins of gastric juice. We have tried to add to the results obtained by these methods by using electrophoresis in agar[1] and immuno-electrophoresis[2]. With these methods, especially in the case of immuno-electrophoresis, we found that there exists a fundamental difference in the behaviour of acid and anacid gastric juices.

Agarophoresis. The electrophoretograms of fifteen anacid gastric juices (*p*H 4–7) of patients with histamine-resistant achlorhydria showed five protein fractions, the mobility of which corresponded to that of serum proteins (Table 1, Fig. 1*a*). In three anacid gastric juices where all subsequent portions contained free hydrochloric acid after stimulation by histamine, two additional zones faster than albumin were observed. The electrophoretic mobility of this pre-albumin is identical with that of crystalline pepsin.

Table 1. Protein Fractions of Gastric Juice after Electrophoresis Separation in Agar

Samples	Pepsin 2 ×	Albumin	Globulins α_1	α_2	β_1	β_2	γ
15 anacid gastric juice	—	15	—	7	15	15	12
3 anacid gastric juice	3	3	1	3	3	3	3
10 anacid gastric juice	10	10	2	8	6	10	—

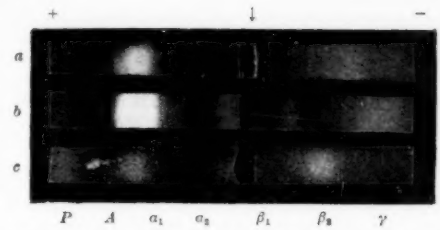

Fig. 1. Electrophoresis in agar: *a*, anacid gastric juice; *b*, serum; *c*, acid gastric juice

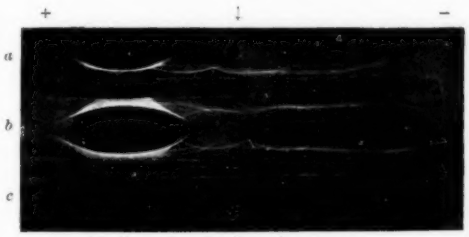

Fig. 2. Immuno-electrophoresis: *a*, anacid gastric juice; *b*, serum; *c*, acid gastric juice

In ten acid gastric juices seven fractions including two pre-albumin zones of pepsin were noted (Table 1, Fig. 1*c*).

Immuno-electrophoresis. The proteins of 22 anacid gastric juices precipitated on immuno-electrophoretic plates by rabbit antihuman serum developed 3–10 precipitation lines (Fig. 2). Their location both in the direction of electrophoresis and of diffusion corresponded to the precipitation lines of blood serum. In the double diffusion test[3] the albumin of the gastric juice gave the identity reaction with serum albumin. γ-Globulin reacted likewise with γ-globulin antiserum. The precipitation line of β_2-globulin was only about 30 per cent determined, although a zone of strong density was observed in all gastric juices by simple agarophoresis. This indicates that in all anacid gastric juices besides β_2-globulin, which is not precipitated by antihuman serum and thus differs immunologically from serum proteins, an inconstant serum β_2-globulin in lower concentration is present.

The proteins of ten acid gastric juices were not precipitated with antihuman rabbit serum. It follows from our investigation on the influence of single components of gastric juice on serum proteins that the protein fractions lose their immunochemical properties by the action of the activity of pepsin and the acid medium in dependence on time.

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Attachment of Antibody to Influenza Virus

A KINETIC analysis of the neutralization of influenza virus by rabbit antibody indicated that the combination proceeds in two stages[1]. The first stage is the formation of a freely dissociable union between the virus and antibody. This is followed by a second stage which leads to the stabilization of the complex between the virus and antibody. The reaction kinetics also indicated that a single antibody molecule could form a stable combination with the virus particle. In an attempt to elucidate the nature of the stage of stabilization, the interaction between influenza virus and the univalent antibody fragments, produced by papain digestion[2], was investigated[3]. This investigation demonstrated that although the monovalent fragments could combine reversibly with the virus and neutralize its infectivity, they were unable to form a stable combination with the virus. It could be concluded that the stabilization of the combination between the virus and antibody depended on the divalent nature of the antibody molecule. On the basis of the foregoing conclusions and observations the following hypothesis emerged. The first stage in neutralization would be the attachment of the antibody molecule to the surface of the virus through one combining site and the second and stabilizing stage would be brought about by the attachment of the second combining site on the divalent antibody molecule to the same virus particle.

This hypothesis was supported by the finding that the fraction of virus particles which could undergo the stabilization reaction declined sharply when the reaction was studied in the region of extreme antibody excess[3]. This would be expected for the following reason. The stabilization stage proceeds at a much slower rate than the first stage of the interaction between virus and antibody. Thus, when the virus is mixed with high concentrations of antibody, antigenic sites on the surface of the virus particle will be rapidly occupied by antibody molecules attached through only one combining site. The combining sites of the virus will be saturated with antibody and the neighbouring molecules may sterically hinder the attachment of any antibody molecule to one and the same virus by both its combining sites. Indeed, work on the interaction between tobacco mosaic virus and antibody, carried out with the aid of the electron microscope, demonstrates that in the region of antibody excess the antibody molecules are attached to the virus surface through one combining site, with their long axes radiating out from the surface of the particle[4,5]. A similar type of combination has been observed in these Laboratories in work on the interaction between influenza virus and a large excess of antibody.

The kinetic evidence demonstrated the occurrence of a reaction of stabilization, but could not provide detailed information on the mechanism of the stabilization reaction. The following experiment was therefore conducted in an attempt to obtain more direct information on this point. Purified influenza A virus (*MEL*) was mixed with a solution of the globulins obtained from an *MEL* virus rabbit antiserum and held at room temperature for 4 hr. The concentration of the virus in the mixture was 4×10^4 hæmagglutination units/ml. and the concentration of antibody was 300 hæmagglutination inhibition units/ml. Virus and antibody titrations were carried out as described by Fazekas de St. Groth *et al.*[6,7]. The complexes of virus and antibody were sedimented in the ultracentrifuge, washed with saline and finally resuspended in distilled water. As a control, a preparation of *MEL* virus that had not been mixed with antibody was treated in the same way as the mixture of virus and antibody.

Specimens of the two suspensions were prepared for electron microscopy by mixing equal volumes of the suspensions and a 1 per cent solution of sodium phosphotungstate[8]. A drop of this suspension was allowed to dry on carbon-coated 'Formvar' membranes and the specimens were observed in the electron microscope (Siemens Elmiskop I). Fig. 1*a* shows the morphology of the virus particle and Fig. 1*b* the type of complex formed when the virus reacts with antibody. It can be seen that the antibody molecules become attached to the terminal ends of the sub-units which constitute the surface of the virus. The antibody molecules appear to be attached to the individual virus particle through both sites situated at the extremities of the antibody molecule, thus forming a loop on the surface of the virus particle.

It is known that the two combining sites on an antibody molecule have identical specificities[9,10]. This may well imply that the combining strength at each site is very similar. If this were so, the combination of the antibody with a virus particle through both combining sites of the antibody molecule would be much more stable than the combination through only one site, provided the strain introduced by the bending of the molecule did not significantly reduce the strength of the bonds formed through each combining site. The fact that a two-site attachment rather than a single-site attachment is produced when the virus and antibody react under conditions where the antibody is not in very large excess indicates that the former type of attachment is more stable than the latter.

Thus, the observation of two-site combination between virus and antibody provides a physical mechanism which would account for the stabilization reaction.

A more detailed publication demonstrating the effect of the concentration of the reactants on

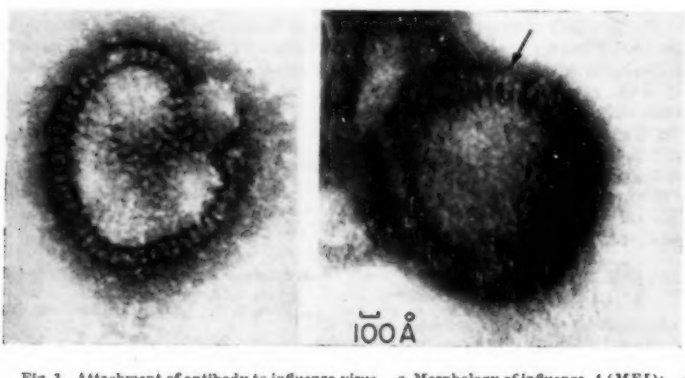

Fig. 1. Attachment of antibody to influenza virus. *a*, Morphology of influenza A (*MEL*); *b*, combination of *MEL* virus with *MEL* virus antibody

the type of combination formed is in preparation.

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HÆMATOLOGY

Absence of Red Cell Enzyme Deficiency in Australian Aborigines

The geographical distribution of erythrocyte glucose-6-phosphate dehydrogenase deficiency is now known to be widespread. Thus, this genetically determined enzyme defect has been shown to be present in parts of Africa, Asia and Europe, and in some Indian groups in South America[1]. Marked variations in the incidence of the defect have been observed between different populations and in some instances between different groups in the same population. In south-east Asia recent work has shown the trait to be present in Thais[2], Malays[3a], Indonesians (Lie-Injo, L. E., personal communication), Filipinos[3] and Melanesians[4].

In the present work, using the rapid screening test described by Motulsky[5], a total of 722 Australian Aborigines were examined for evidence of glucose-6-phosphate dehydrogenase deficiency. The subjects tested live in scattered areas of northern, central and western Australia. All have tribal affiliations. The groups studied are shown in Table 1.

Table 1

Area	Males	Females	Total
Cape York Peninsula			
Edward River	43	45	88
Mitchell River	84	62	146
Lockhart River	27	—	27
Aurukun	39	37	76
Weipa	12	29	41
Wrotham Park	12	11	23
Central Australia			
Alice Springs	140	—	140
Western Australia			
Port Hedland	40	59	99
Marble Bar	11	13	24
Nullagine	27	31	58
Total	435	287	722

No cases of enzyme deficiency were found in this large population sample, indicating that the gene frequency is extremely low or non-existent. This finding is of interest in several respects. Except for occasional cases of malaria introduced in recent times from surrounding areas Australia is malaria-free. The incidence of red cell enzyme deficiency has been shown, in Africa[3,6], New Guinea[4,7] and Sardinia[8], to be related to that of falciparum malaria, probably representing a selective advantage by way of increased resistance to malarial mortality. Absence of the deficiency in Australian Aborigines supports this concept, as there would be no such advantage enhancing the survival of affected people in Australia.

Glucose-6-phosphate dehydrogenase deficiency has an incidence as high as 20 per cent in males of coastal New Guinea[7]. The penetration of the blood group *B* gene in Aborigines of the Cape York Peninsula groups is accepted as representing admixture from New Guinea[9]. It is evident, however, that this penetration has not resulted in the introduction of the enzyme deficiency, or, if the gene has been introduced in the past, it has not survived. That the well-known Malay contact with Aborigines in several areas of the north and north-west coast has not resulted in the introduction of the trait is not surprising, since its incidence in Malays is low, probably less than 1 per cent.

Absence of this deficiency of red cell enzyme, then, adds important evidence to that already accumulated, including the absence of thalassæmia and the abnormal hæmoglobins, which demonstrates the degree of isolation of the Aborigines from surrounding peoples. This trait will serve as an additional link in the chain that may eventually disclose the origin of the Australian Aborigines.

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Distribution of Haptoglobin Types in Israel

The interest in the patterns of haptoglobin types has been furthered during the past several years due to the introduction of starch-gel electrophoresis for their demonstration[1]. Different populations have been investigated up to now[2,3].

Since differences between Jewish ethnic groups in Israel were described in relation to blood group patterns[4], incidence of erythrocyte glucose-6-phosphate dehydrogenase deficiency[5], and taste sensitivity to thiourea (Sheba, Ch., Ashkenazi, I., and Szeinberg, A., personal communication), it was of interest to investigate the haptoglobin patterns among them.

Blood specimens from 526 blood donors from the blood bank of Tel Hashomer Hospital, and from patients without any serious illness, were examined. The material consisted of 170 Ashkenasi Jews (East European origin), 118 Iraqi Jews (Babylonians), 104 North African Jews (Morocco, Tunisia, Algeria and

Lybia) and 134 mixed population. The method used was vertical starch-gel electrophoresis as described by Smithies[6]. The starch was sliced longitudinally into two parts; one was stained with benzidine, the other with brom-phenol blue or amido-black 10 *B*. To the sera 120–150 mgm. per cent of oxyhæmoglobin was added to saturate the haptoglobins completely. The results are presented in Table 1.

Table 1. HAPTOGLOBIN TYPES AND FREQUENCY OF ALLELE Hp^1 AMONG DIFFERENT ETHNIC GROUPS IN ISRAEL

Group	No. tested	*Hp* 1 : 1 No.	*Hp* 1 : 1 Per cent	*Hp* 2 : 1 No.	*Hp* 2 : 1 Per cent	*Hp* 2:1 *M* No.	*Hp* 2:1 *M* Per cent	*Hp* 2 : 2 No.	*Hp* 2 : 2 Per cent	Gene frequency Hp^1
Ashkenazi	170	13	7·65	88	51·76	—	—	69	40·59	0·34
Iraqi	118	9	7·63	50	42·37	—	—	59	50·00	0·29
North African	104	8	7·69	43	41·35	1	0·96	52	50·00	0·29
Rest of population	134	13	9·70	51	38·06	—	—	70	52·24	0·29

No cases of ahaptoglobinæmia were found except in hæmolytic anæmias, which are not included in this work. Only one case of 2 : 1 *M* was found, that being among the North African Jews. The gene frequency of Hp^1 in Ashkenazi Jews was found to be 0·34, while that found among the Iraqi and the North African Jews was 0·29. This difference is not statistically significant. The expected values of phenotype were calculated on the basis of the Hardy–Weinberg equilibrium and were found to correspond closely to the observed numbers.

Our results show that the frequency of Hp^1 among the Ashkenazi Jews is similar to that found in European populations[2], while the Iraqi and North African Jews have a lower frequency of Hp^1 similar to that found in Asian populations[3]. Other Jewish and Arab populations are under investigation.

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Distribution of Group-specific Components (Gc) in the Sera of Native Africans

THE group-specific components of sera consist of electrophoretically variable α_2-globulins demonstrable by means of an immuno-electrophoretic technique[1]. This system allows a division of human sera into three qualitatively different patterns characterized by the presence of a fast-moving group-specific component (*Gc* 1–1), a slow-moving component (*Gc* 2–2), which in immuno-electrophoresis both give normally arc-shaped precipitates, or the simultaneous presence of both fast- and slow-moving components (*Gc* 2–1), which in immuno-electrophoresis give an extended two-peaked precipitate due to a reaction of immunological identity between these electrophoretically different although immunologically identical fast- and slow-moving components[1]. Investigations on sera from 42 families with 137 children and more than 600 mother–child combinations have shown that these serum patterns are genetically determined by two autosomal alleles (Gc^1 and Gc^2) without dominance where the serum types *Gc* 1–1 and *Gc* 2–2 are the homozygotes and *Gc* 2–1 the heterozygote. Up to now, no exceptions to this genetical theory or unresolved typing difficulties has been found in the material investigated[2,3].

Table 1. FREQUENCIES OF THE Gc^1-GENE IN VARIOUS POPULATIONS

Population	No. tested	Gc^1	*Gc*-determinations carried out by:
Sweden	1,744	0·74	Hirschfeld*
Swedish Lapps	79	0·87	Hirschfeld*
New York Whites	86	0·74	Bearn and Cleve†
New York Negroes	120	0·90	Bearn and Cleve†
Navajo Indians	197	0·98	Bearn and Cleve†

* Hirschfeld, J., and Beckman, L., *Acta Genet.*, **11**, 185 (1961).
† Personal communication.

Table 2. DISTRIBUTION OF *Gc*-TYPES IN SERA FROM 100 AFRICANS

	Gc 1–1	*Gc* 2–1	*Gc* 2–2	Gc^1
obs.	82	17	1	0·91
exp.	81·9	17·2	0·9	

The frequencies of the Gc^1-gene in populations hitherto investigated are given in Table 1. In Table 2, the distribution of *Gc* in a serum sample consisting of sera from 100 native Africans collected in Léopoldville, Congo, are given. The results seem to show that the system might be of value in connexion with anthropological investigations, and further work concerning the distribution of *Gc*-types in different populations is in progress.

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Note added in proof. A more detailed survey of the distribution and inheritance of the *Gc*-system will be given elsewhere[3].

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RADIOBIOLOGY

Autoradiographic Study of Cell Migration during Histogenesis of Cerebral Cortex in the Mouse

AUTORADIOGRAPHY with thymidine labelled with tritium has proved useful for the study of histogenesis in the mammalian brain[1–6]. Labelled thymidine injected into animals becomes incorporated almost exclusively into deoxyribonucleic acid of cells preparing to divide and remains in their nuclei as a permanent label; the radioactivity is diluted to half with each cell division[7]. When the autoradiographic method is applied to tissues of animals killed serially, one can determine the time and place of origin of cells, their route and rate of migration, and their ultimate fate.

The question has been raised as to whether cells which appear to change position during development are truly migrating or are merely being displaced by cells which form at subsequent stages. The present work demonstrates that migrations of cells do indeed take place in the developing brain.

Tritiated thymidine (5 μc./gm. of body-weight) was injected once intravenously into pregnant mice. The animals were injected in pairs on the 11th, 13th, 15th, or 17th days of gestation. One animal of each pair was killed 1 hr. after injection, and offspring of the other injected animal were killed on the 10th postnatal day. Brains were processed for autoradiography as described elsewhere[1].

In an embryo killed 1 hr. after injection on the 11th day, the telencephalic vesicle consists only of a primitive ependymal zone; most of its cells are labelled, as has been observed previously[1]. Many of the primitive ependymal cells later migrate outward to form the anlage of the cortical plate. In embryos injected on the 13th, 15th, or 17th day of gestation and killed 1 hr. later, the cerebrum shows labelled cells almost exclusively in the primitive ependyma and relatively few in the developing cortical plate. Thus, the source of most cortical cells is the primitive ependyma of the lateral ventricles. After mitosis in the primitive ependyma, cells migrate outward to form the cortical plate and differentiate into its constituent neurons.

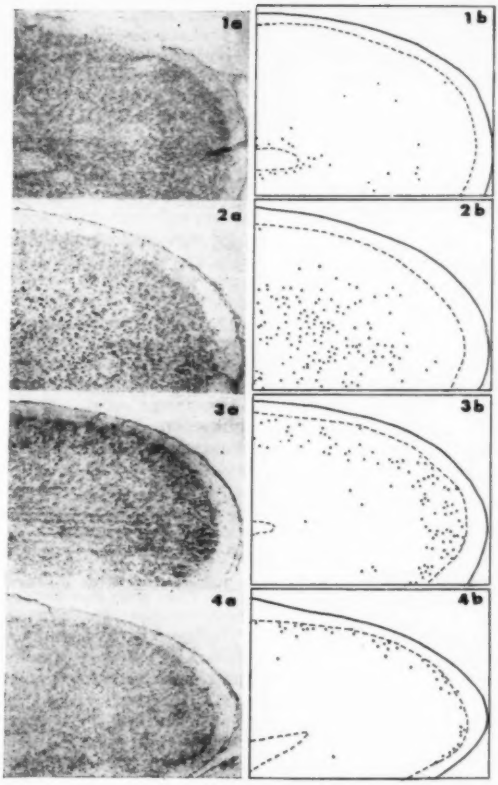
To study the postnatal disposition of cortical cells arising in the 11-, 13-, 15-, and 17-day embryos, we photographed the entire anteroposterior extent of the cerebral cortex of the corresponding four 10-day old mice. For each brain a series of overlapping photomicrographs was made at a magnification of 150 diameters on 4 × 5 in. sheet film, and prints were assembled as a montage to show the distribution of labelled cells. Comparable sections of occipital cortex from the 4 mice are illustrated in Figs. 1–4 and the following description refers to this region of cortex.

Many cells labelled on the 11th day of gestation undergo only 1 or 2 further divisions and are recognized in the brain of the 10-day old animal as heavily labelled cells. These cells are almost exclusively in the deepest part of the cortex adjacent to the white matter (Fig. 1). Other cells labelled on embryonic day 11 divided on subsequent embryonic days and appear in the 10-day old mouse as weakly labelled cells scattered through all cortical layers.

The mouse labelled on the 13th day of gestation and killed 10 days after birth shows a greater number of heavily labelled cells; these are located predominantly in the middle third of the cortex (Fig. 2). These cells originated later than the cells which arose on the 11th embryonic day and migrated past their predecessors upon entering the cortical grey matter.

The same pattern of origin and migration obtains for cells forming later in gestation. Cells which arise on the 15th embryonic day migrate past cells which had formed earlier and come to lie in the outermost third of the cortex (Fig. 3). Relatively few cells arising on the 17th embryonic day are destined for the occipital cortex; these migrate to the most superficial cell layer, the presumptive lamina ii (Fig. 4).

As Figs. 1–4 show, cells originating on any particular embryonic day come predominantly but not exclusively into a given cortical level. Cells forming successively later in embryonic life migrate outward past their predecessors and come to lie at more and more superficial levels. The correlation between these levels and cortical laminæ as conventionally designated will be considered in a more complete account of cortical histogenesis now in preparation.



Figs. 1–4. Photomicrographs (*a*) and matching drawings (*b*) of comparable parasagittal sections of occipital cortex from four 10-day-old mice injected with thymidine labelled with tritium on the eleventh, thirteenth, fifteenth and seventeenth days of gestation respectively. Dots in drawings indicate heavily labelled cells. Intensity of labelling in photomicrographs varies because tissues were exposed to the emulsion for different durations prior to development of the autoradiograms. Outer broken line represents inner boundary of molecular layer of cortex and inner broken line the boundary between cortex and white matter. (× *c.* 38)

Cortical zones other than occipital cortex display a similar pattern of histogenesis. The times of origin and migration of cells into other cortical regions differ from the times described for occipital cortex.

If cells do not migrate outward, but are merely displaced peripherally by new cells which arise afterwards in the primitive ependyma, one would expect the more superficial layers of cortex to form first and the deep layers last, as has been suggested[8]. Our autoradiographic results indicate just the reverse, and show that cells do migrate extensively in the developing mammalian cerebrum.

This curious reverse order of formation of cortical layers may have significance for the attainment of intracortical synaptic connexions. It is recognized that the axons of most cortical neurones are directed inward toward the white matter and that their dendrites are directed outward[9]. The orientation of the circuits established by synaptic contact of these processes is predominantly perpendicular to the plane of the cortex[10]. These synaptic contacts might be attained during the histogenetic period, as cells migrate past those neurones that have arrived pre-

viously in the developing cortex. The perikaryon of a cell migrating outward may perhaps establish contact with another cell already in position in the cortex and may then migrate farther while trailing its axon behind, still in contact with the perikaryon or the developing dendritic tree of the cell that had arrived previously. The cell contacts established during histogenetic migration may also be relevant for the formation of particular patterns of synaptic connexions in other parts of the nervous system, as discussed elsewhere[3,4].

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Uptake and Incorporation of Tritiated Thymidine in *in vitro* Culture

The duration of the 'synthesis phase' of deoxyribonucleic acid (DNA) of the mitotic cycle (phase *S* of Howard 1956[1] and Lajtha *et al.* 1954[2]) has been determined for several cell types. As yet no distinction has been made between the uptake of labelled thymidine and its incorporation by the cell into a more complex structure, presumably DNA.

Sagittal slices of the upper tibial epiphysis of the rat, rabbit and guinea pig were cultured *in vitro*, in the presence of tritiated thymidine (0·5–1 μc. tritiated thymidine, specific activity 0·360 c./m.mol., to 5–6 ml. medium), in an attempt to elucidate the growth pattern of the epiphyseal growth cartilage and the articular cartilage. The explants were fixed in 10 per cent formalin at *p*H 7·4–7·6. Some were embedded in butyl and isobutyl methacrylate and sectioned without decalcification; others were first decalcified with 10 per cent formic acid for 12 hr. before being embedded in paraffin wax. Sections 4–7μ thick were cut, the embedding medium removed, and autoradiographs made according to the Pelc stripping film technique[3], and exposed for 3–6 weeks. Grain counts were made on cells in the proliferative zone (flattened cells) of the epiphyseal growth cartilage.

If the tissue was sectioned without previous decalcification, the grain counts attained nearly the maximum value after ½ hr. in culture. By 1 hr. the maximum value was found; this persisted for the duration of the experiment (Fig. 1).

On the other hand, if the tissue was first decalcified with formic acid, maximum counts were only attained on explants that had been cultured for 6–9 hr. (Fig. 2, rat). Similar results were obtained by giving the tritiated thymidine to rats *in vivo*.

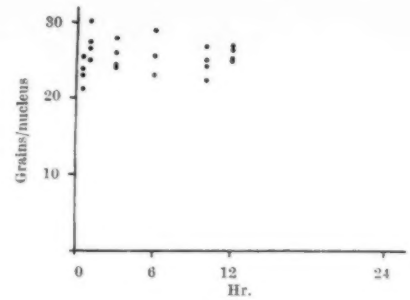

Fig. 1

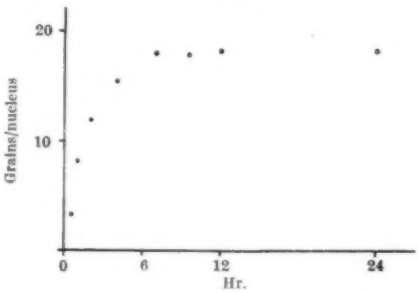

Fig. 2

These results suggest that a maximum load of tritiated thymidine is taken into the cell nucleus very rapidly. In fact, this occurs so rapidly, in less than 1 hr., that a carrier mechanism might have to be invoked to explain it. But once in the nucleus, it takes a finite time for the tritium-labelled substance to be incorporated into a large and more complex molecule capable of resisting removal by 10 per cent formic acid. This process of incorporation would appear to take between 6 and 9 hr. to be completed in the cells of the proliferative zone of the epiphyseal growth cartilage.

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BIOLOGY

Fluctuations in Marine Populations in Icelandic Waters

It is well known that populations of many animals living at high latitudes are subject to considerable fluctuations. Fairly regular periodic variations are reported in a number of cases. The 10-yr. cycle of the rock ptarmigan in Iceland reported by Gudmundsson[1] furnishes a striking example. The cause of this cycle is unknown.

Marine populations are also subject to large fluctuations but there are few reports on regular or periodic variations. This may in many cases be due to lack of observations.

There are available fairly reliable data on the catching of cod in the waters south-west of Iceland since 1900. In particular, there is available a set of data of a rather uniform quality for the winter

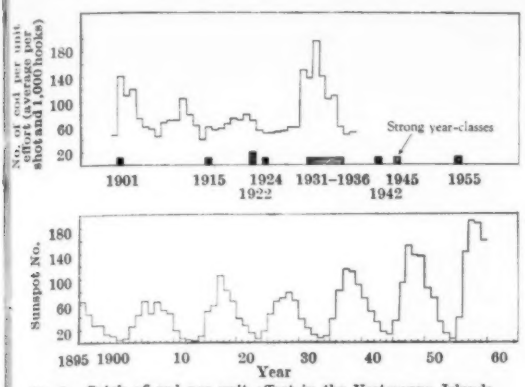

Fig. 1. Catch of cod per unit effort in the Vestmanna Islands during 1900–40, and its relation to observed strong year-classes and the sunspot number

seasons during 1900–40. Data on the catch per unit effort have been collected in the Vestmanna Islands and are given as the yearly average number of cod caught per shot and 1,000 hooks. The data are given in Fig. 1. Unfortunately these data have not been collected for the period since 1940.

The catch per unit effort given in Fig. 1 is subject to a rather regular variation with a period of approximately 10 yr. This happens to be the same period as that of the rock ptarmigan. There is little doubt that the catch per unit effort depends largely on the number of individuals per unit volume, and the data therefore give a certain measure of the variation in the size of the spawning stock.

The fluctuations in the strength of the spawning stock of cod are due to pronounced differences in the strength of the individual year-classes. Certain year-classes prevail in the commercial catch. The recruitment age for more than 80 per cent of the stock is 7–10 yr.

Reliable data are available on the strength of individual year-classes since 1920. Moreover, it is known that the 1901 and 1915 year-classes were relatively strong although very little is known about other year-classes during 1900–20.

The prevailing year-classes are given in Fig. 1. There appears to be a tendency for an 11-yr. period in the emergence of strong year-classes. The class of 1915 is somewhat anomalous, and the period 1931–1936 is characterized by a series of medium-size classes. This, however, does not interfere with the rather regular pattern.

For several decades it has been repeatedly pointed out that certain biological phenomena appear to exhibit periodicities related to the 11-yr. solar cycle. In most cases, however, the evidence has been very weak and there have been no reliable indications as to the relation between the solar and the biological phenomena.

Quite recently, however, it has been pointed out by MacDonald and Roberts[3] that solar corpuscular emission may affect the surface weather in a significant way. Also, Landsberg *et al.*[4] have been able to detect a weak tendency for a sunspot rhythm in the temperature of the United States.

In view of these circumstances we have compared the emergence of strong year-classes of the Icelandic cod with the sunspot rhythm. The Zurich sunspot number has been plotted in Fig. 1. The results at hand indicate quite definitely that the strong year-classes emerge preferably during periods of low solar activity.

In order to extend these observations we have studied the available reports on the catch of other species in Icelandic waters. Unfortunately these reports are much less reliable than the above data on the catches of cod. However, the results are not altogether negative.

The catch of herring during the summer season in the waters north of Iceland, measured in volume per boat, exhibits relative maxima in 1923, 1933 and 1944. These are years of low solar activity. Moreover, the total catch of salmon in the rivers of Iceland, measured in number of salmon per annum, exhibits pronounced maxima in 1913, 1922 and 1933, which are years of low solar activity. On the other hand, the available data on the total catch of lumpfish in Icelandic waters exhibits maxima in the periods of high solar activity. Data on lumpfish are at hand for 1915–50. The maxima correlate moderately well with the solar maxima of 1917, 1928 and 1938.

A discussion of possible causes of the more or less apparent correlation between the solar rhythm and biological phenomena is beyond the scope of this communication. We realize, of course, that the fluctuations of the populations may be due to some local or internal cause and the relation to the solar rhythm only accidental.

In order to look for a climatic factor we have made a preliminary investigation of the solar-weather relationship for Iceland during 1900–60. The fluctuations in the annual and the monthly means of the temperature and the precipitation in Reykjavik have been investigated. The results are negative so far. No relation to the solar rhythm has been detected. However, we wish to emphasize the preliminary nature of this work.

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Colour and Density of Light and Broiler Performance

It has been shown that some form of artificial lighting is necessary to achieve the most rapid gains of weight in broilers[1]. With controlled environments, light intensities of 0·1–6·0 ft.-c. gave equally good growth-rates in broiler chicks during the first eighteen days, and 12·0–24·0 ft.-c. produced slightly depressed growth-rates during this period[2]. A field scale survey has noted that in many broiler houses in Britain the light intensity is maintained at 0·05–0·20 ft.-c. as a control measure for the prevention of cannibalism[3]. An experiment is being conducted which has already compared three types of continuous lighting in relation to broiler performance.

Six groups, each of 111 Rhode Island Red × Light Sussex cockerels hatched on August 27, 1960, were reared under identical conditions except for variations of light in environmental chambers 14 × 8 ft. Two groups served as controls and their chambers were each lighted by two 15-W. white incandescent

Table 1. Water Consumption, Mortality and Growth to 7 Days of Age

Lighting treatment	Mean water consumption (pints) per pen First 3 days	First 7 days	per survivor First 7 days	No. mortalities per pen during first 7 days	Weight increases per chick during first 7 days (oz.)
Controls	11·2	35·5	0·323	1·0	2·47
Dull red	9·2*	30·0	0·284	5·5*	2·12*
Dull white	10·5	34·0	0·313	2·5	2·26
Least difference to be significant between two means, $P < 0.05$	1·0	4·3	N.S.	1·9	0·15

N.S., no significant difference

* Significantly different from the control; $P < 0.01$

Table 2. Average Results to 72 Days of Age

Lighting treatment	Mean No. mortalities per pen	Live-weight (lb.) Total per pen	Average per broiler	Food consumption (lb.) Total per pen	Average per broiler	Food efficiency (food consumed per unit of live-weight gain)
Controls	1·0	402·7	3·661	1085·7	9·87	2·696
Dull red	7·5*	367·6*	3·552	976·0*	9·43*	2·655
Dull white	3·5†	387·8†	3·607	1009·5*†	9·41*	2·603*
Least difference to be significant between two means $P < 0.05$	1·9	10·6	0·101	3·3	0·21	0·043

* Significantly different from the control; $P < 0.01$;

† Significantly different from the dull red treatment; $P < 0.01$

bulbs. Two further pens each had two 40-W. red bulbs and the remaining two had two 5–8-W. white economy bulbs. Lighting was continuous, and the intensities recorded were 0·20–0·50 ft.-c. in the control pens, and 0·05–0·10 ft.-c. in the other four pens which were lighted by dull white and dull red bulbs.

During the first few days the form of lighting affected the amount of water consumed. This is shown in Table 1. Differences in mortality and growth-rates to one week of age were also apparent. The use of dull red lighting was associated with increased mortality compared with the control ($P < 0.05$). At the end of the first week the controls were heavier per survivor than the birds of either of the two experimental groups ($P < 0.05$ dull white lighting; $P < 0.01$ dull red lighting).

Table 2 shows the results to 72 days of age. The control chicks ate significantly more per pen and more per bird than did either of the two experimental treatments. Although the total live-weight of the controls was greater than that of either of the other two experimental groups ($P < 0.05$ dull white lighting; $P < 0.01$ dull red lighting), the average live-weight of the controls was only significantly heavier than the broilers reared under the dull red lighting. These variations were partly due to differences in mortality. The birds receiving dull white light had an improved food conversion factor compared with the control ($P < 0.01$) and compared with those receiving the dull red treatment ($P < 0.05$). No cannibalism was observed under any treatment, and there was no downgrading because of stubby, undeveloped feathers when the broilers were sold.

This experiment, which is being extended, is conducted with the aid of grants and equipment from the Agricultural Research Council, Calor Gas, Ltd., Electrical Development Association, Fenton Byrn, Ltd., Maywick Appliances, Ltd., and Wernick Construction Co., Ltd., in the Department of Animal Husbandry at the Royal Veterinary College, London.

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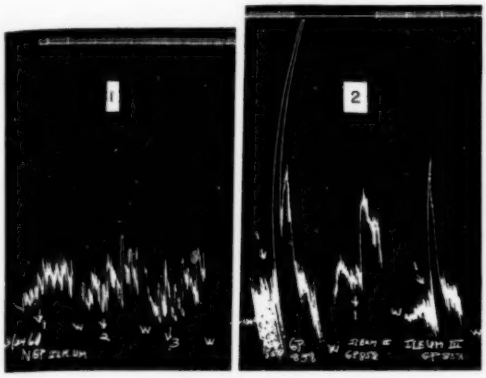
Antigenicity of Guinea Pig Testicular Cells grown in Cell Culture as determined by the Isolated Ileum Technique

I have previously recorded experiments relating to the antigenicity of testicular tissue and of sperm as well as extracts thereof[1]. For many reasons, for example to facilitate identification of spermatogenic tissue antigens and to elucidate the mechanism(s) of cell destruction occurring in immunologically induced aspermatogenesis, it was important to have replicating cell cultures of testicular tissue. Replication, rather than mere maintenance, of testicular cells in culture has recently been accomplished, and the results will be published *in extenso* elsewhere. The attainment of this goal imposed the requirement of demonstrating that spermatogenic cells grown in culture continue to synthesize antigens that are present in the germinal epithelium of the testicle *in vivo*. Among such antigens are those capable of: (1) eliciting anaphylactic contracture of sensitized ilea; (2) inducing aspermatogenesis; (3) provoking the production of humoral antibody. We have found that replicating guinea pig cells carried in culture for more than 2 months are capable of causing anaphylactic contracture of sensitized ilea and can also be used to sensitize guinea pigs.

Eight adult male guinea pigs (600–700 gm.) were injected with guinea pig testicular homogenate in Freund's adjuvant. The preparation of the adjuvant-antigen mixture and the method of injection have already been described[1]. Briefly, a weighed amount of testicular tissue is homogenized in an equal volume of 0·9 per cent sodium chloride solution and the homogenate is then incorporated into an equal volume of the adjuvant so as to form a stiff gel. This preparation is injected intracutaneously in 6–8 sites in the nuchal region of the male guinea pigs. Three weeks after injection, 4–5-cm. segments of the ileum are removed from these animals and suspended in an oxygenated bath of Locke–Ringer's solution. When challenged with specific antigen (homologous sperm or purified extracts thereof), these segments contract anaphylactically. This test has been found to be a consistently reliable indicator of the presence of antibody to spermatogenic antigen(s). In the present work, therefore, the presence of spermatogenic antigen(s) in the cell cultures of the guinea pig testicular tissue was determined by: using the cell



Figs. 1 and 2. (1) An ileal segment of a non-sensitized guinea pig is not caused to contract when challenged with three different cell cultures of (at 1, 2 and 3, respectively) guinea pig testis. (2) Ileal segments from a guinea pig sensitized with homologous testis homogenate contract anaphylactically when challenged with the same three cell cultures noted in Fig. 1

cultures to challenge ileal segments of guinea pigs sensitized with whole testicular homogenate and by using homologous sperm to challenge ileal segments of guinea pigs sensitized with the cell cultures of guinea pig spermatogenic tissue.

Experimental Series I. Ileal segments from non-sensitized guinea pigs were suspended *in vitro* and challenged with three different cultures of guinea pig testicular cells that were collected 2 months following initiation of the cultures. The cells were separated from the culture media by centrifugation and were suspended in Locke–Ringer's solution (1 ml.: 0·5 ml. of packed cells) so that approximately $3{\cdot}3 \times 10^7$ cells were present per ml. of fluid. The cells were then homogenized in a glass hand homogenizer and 1·0 ml. of the homogenate was added to the bath fluid containing the ileum. Responses of the segments of ileum were recorded with the aid of a kymograph.

Experimental Series II. Segments of ileum from guinea pigs sensitized with homologous testicular homogenate were challenged with the following: (1) homologous spermatozoa; (2) the three preparations of cell cultures used in experimental series I (above); (3) a concentrate of the tissue culture media separated from the cultured cells by centrifugation.

Experimental Series III. Eight male guinea pigs were injected with the three cell cultures homogenized in saline and incorporated into Freund's adjuvant. Three weeks later, segments of the ilea of these animals were challenged with homologous sperm.

The homogenized cultured spermatogenic cells did not cause contracture of ileal segments from non-sensitized guinea pigs. This is illustrated in Fig. 1, a kymograph tracing demonstrating that not one of the three preparations (injected into the bath at 1, 2, and 3, respectively) affected the tonus of the segment. Moreover, the media in which the cells were grown did not cause contraction of ileal segments from non-sensitized or sensitized animals.

In sharp contrast are the responses of ileal segments of sensitized animals when challenged with the same three cell culture preparations. In Fig. 2 it is apparent that each of the cell homogenates caused a strong contracture (at 1, 2, and 3, respectively) of an ileal segment from a guinea pig injected with whole testicular homogenate. Similar responses were obtained when ileal segments from animals sensitized with the cell culture homogenates were challenged with homologous sperm.

The results of these experiments indicate that guinea pig testicular cells grown in culture for more than 2 months contain antigens that can sensitize ilea and that elicit contracture of the ileum of sensitized guinea pigs. The antigens were not carried over from the original cells because the final cultures represent seven serial transfers and it is calculated that few, if any, of the original cells could have been present in the final populations employed. Thus, antigen synthesis *in vitro* must have occurred. These observations now make it possible to examine more definitely the problem of elucidating the mechanism(s) of cell destruction occurring in immunologically induced aspermatogenesis.

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Cell Reduction in the Embryonic Development of the Cestode *Diorchis ransomi* Schultze, 1940

During work on the embryonic development of the cestode *Diorchis ransomi* Schultz, 1940, considerable attention was paid to the final phase called the pre-oncosphere. This phase is relatively little known so far. The present communication deals with only one feature of the pre-oncosphere, namely, the reduction in number of embryonic cells. A more detailed account of the work will be published in *Acta Parasitologica Polonica*.

The embryogenesis was investigated in 5μ-thick serial sections of gravid segments. The staining methods used were: Prenant's trichrome, Feulgen technique, Brachet technique and toluidine blue with a control hydrolysed by hydrochloric acid. As a result of the reconstruction of particular embryos from serial sections I was able to establish with reasonable accuracy the number of cells in subsequent developmental phases.

In the early embryos three types of cells were recognized: macromeres, mesomeres and micromeres. They were identified by their dimensions and nuclear structures. The mesomeres formed the outer membrane of the embryo. The other cells multiplied very intensively, the maximum number being 75–80. The majority of them were micromeres. The cytoplasm of early embryo cells was a syncytial one and ribonucleic acid (RNA) was shown to be present after treatment with the technique of Brachet.

The pre-oncosphere phase began after the formation of the outer membrane. Besides the other morphological and cytochemical changes, considerable cell reduction took place at this phase. The 75–80 cells previously observed in the embryo decreased, finally reaching about 26 in the fully formed oncosphere. At the same time the following cytochemical observations were made. After staining with the Feulgen reagent, two types of micromere nuclei were recognized. The first were homogeneous nuclei, which were very numerous, but irregular fragments of decomposed nuclei were also observed. The latter were nuclei with a distinct chromatin

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network and were relatively small in number. Simultaneously, the Brachet technique showed a considerable decrease in RNA in the syncytial micromere cytoplasm, which remained colourless with all the staining methods used. The mesomere nuclei did not show any particular change in the pre-oncosphere phase. The cytoplasm became compact and formed a clearly outlined layer around each nucleus. The Brachet technique and toluidine blue revealed a large increase of RNA content in the cytoplasm. This mesomere structure showed that these are the plastin cells described by Vogel[1].

In the fully formed oncosphere two types of cells were visible. There were 9–13 small nuclei with syncytial colourless cytoplasm in the hook region. About 12–16 plastin cells filled the oncosphere almost entirely. Their strongly basophilic cytoplasm was rich in RNA. Observations of the early metacestode of *D. ransomi* in its intermediate host *Cypridopsis vidua* (Mull.) showed the rapid growth and multiplication of plastin cells. The small nuclei of the hook region disappeared completely after the oncosphere reached the hæmocœl of the host.

In recent papers the only observation known to me on cell reduction in cestode embryos was made by Joyeux and Baer[2] in their work on *Catenotaenia pusilla* (Goeze). They did not, however, deal with the problem in detail.

Ogren[3], in his investigations on *Mesocestoides corti*, Hoeppli, observed the decrease of small cells in the final phase of embryonic development. In his opinion this was a result of the transformation of the small cells into compact plastin cells. The results obtained by me on *D. ransomi* do not seem to confirm Ogren's interpretation. The reduction of the total number of embryonic cells proved that some of the small cells had disappeared. The homogeneous nuclei seen in many micromeres supported this observation. According to recent research on the biological role of RNA (ref. 4) the disappearance of RNA from micromere cytoplasm indicates the lack of developmental potential.

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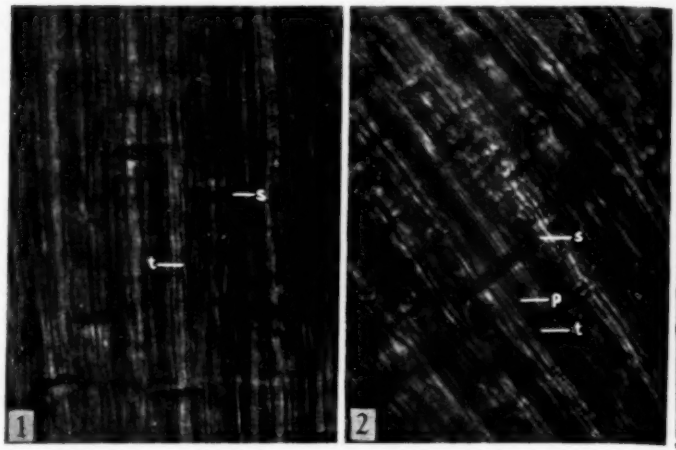

Fig. 1. A fresh hand section of *Primula* petiole phloem showing five sieve plates (*s*) crossed by parallel transcellular strands (*t*). The photomicrograph is an enlargement of a single frame from a 16-mm. ciné film. (× *c.* 1,730)

Fig. 2. A preparation similar to Fig. 1. Transcellular strands (*t*), in which particles (*p*) appear as bright spots and cross a single large sieve plate (*s*). (× *c.* 1,730)

Transcellular Strands and Particle Movement in Mature Sieve Tubes

I have suggested elsewhere[1] that transcellular protoplasmic streaming is the major cause of translocation in vascular plants. The hypothesis depends on the existence of transcellular tubular strands which cross the sieve tube lumina and pass through sieve pores thus providing a structural basis for cytoplasmic communication throughout the vascular system. Within the tubular strands, microscopic structures, comparable with the thread-like constituents of hair cell cytoplasm[1] (and perhaps related to the endoplasmic reticulum seen in electron micrographs of plant cytoplasm[2,3]) are thought to be continuous in the longitudinal direction and to be surrounded by a fluid matrix.

The purpose of this communication is to report the observation of transcellular particle movements along these strands in living mature sieve tubes. Fresh hand sections of *Primula obconica* petiole phloem were mounted in 0·3 *M* sucrose solution and observed in transmitted light with a × 60 apochromatic objective. The observations have been recorded on 200 ft. of 16-mm. ciné film. Figs. 1 and 2 are individual frames taken from the ciné film and show the tissue in which moving particles were seen. Because the particles move within tubular strands they are not sharply defined in such single photomicrographs but they are easily seen in movement in the ciné film and the following statements are based on observations made on the film. Throughout the film, strands (*t*, Figs. 1 and 2) 1·5–2·0μ in diameter are seen in parallel arrangement in sieve tube elements and continuous across sieve plates. Particles (*p*, Fig. 2) can be observed to pass along the lumina of sieve tube elements, and through sieve plates, within transcellular strands and move in opposite directions in single sieve tubes, sometimes within adjacent strands. The particles occur approximately 3μ apart in close linear order in the strands, and their size is comparable with plant mitochondria (diam. 0·5μ). The rate of movement lies within the range 3–5 cm./hr., which is much slower than the translocation-rates recorded in experiments with whole plants, but it is not necessary to assume that particles are the only mobile contents of transcellular strands, and particles may move faster in the stems of plants with long internodes. Moreover, the speed and numbers of moving particles in hand-sections could be affected by slight damage caused in sectioning. The surprising fact is that transcellular particle movement has been observed through as many as ten sieve tube elements (total length 1·0 mm.) in the linear file of a single sieve tube.

in spite of the damage done to transcellular strands at the cut ends of the section.

The particles presumably travel long distances through the phloem and could therefore be involved in transporting mobile materials from the leaf to the stem, the apex or the root. Although particles move past one another in adjacent strands they have not been observed changing direction and are probably part of a two-way transport system. Two-way movement without change of direction implies either that particles originate where transcellular movement begins and are assimilated at their destination or that particles are part of a circulation system in the plant. If the first explanation is correct, particles originating in the stem or root are assimilated in the mature leaf. It is difficult to accept this possibility because leaves export the products of photosynthesis in quantity and import only traces of mobile materials[4-6]. On the other hand, if a circulation system exists in the plant, particles could move out of the leaf loaded with mobile materials and then move back to the leaf after discharging their contents. Circulation streaming seems a more plausible explanation of two-way particle movement in the phloem, and it is interesting that particle circulation is known to occur as part of protoplasm streaming in hair cells.

I wish to thank Prof. Preston for his support and advice during this investigation.

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MICROBIOLOGY

Effect of Ultra-Violet Radiation and Desiccation on Different Age-Cultures of *Escherichia coli K12*

During the course of an investigation on the effect of monochromatic ultra-violet radiation on *E. coli* some unusual observations were made concerning the sensitivity of different age-cultures to ultra-violet light and desiccation. A survey of literature[1-3] revealed conflicting reports on the subject. This work was undertaken to resolve the problem.

For investigating the effect of ultra-violet light on *E. coli* 0·04 ml. (one drop) of suitably diluted culture was spread on a clean sterile glass slide and exposed to ultra-violet radiation for 30 sec. at a distance of 45 cm. from the source. A Hanovia 215-W. quartz mercury lamp housed in an asbestos-lined metal box was used as a source. The exposed slide was then pressed on a nutrient agar Petri dish and gently removed after 15 min. The plates were incubated at 37° C. for 24 hr. This entire operation was carried out in very dim light. After the incubation period the developed colonies were counted. The controls were similarly and simultaneously prepared.

For desiccation work, the glass slides spread with a suitably diluted culture were placed in a metal box and dried at 37° C. in an incubator with the lid slightly open for 3 hr. After this period, the slides were treated as before. The control slides were similarly prepared, but immediately pressed on agar plates without allowing them to dry. These experiments were repeated several times with both glass and stainless steel slides which gave identical results. This eliminates the possibility of attributing the lethal effects to free alkali on the glass surface.

Table 1. Effect of Ultra-violet Radiation and Desiccation on *E. coli*

Age of the *E. coli* culture (hr.)	Percentage viable colonies after drying	Percentage viable colonies after exposure to ultra-violet radiation
0·75	20·8	7·2
1·5	18·5	6·0
3	6·0	2·3
4·5	2·0	1·2
6	4·0	1·5
8	10·0	2·6
12	18·5	3·7
16	20·2	4·0
18	20·1	4·1
24	20·3	4·1

It will be seen from Table 1 that the 0·75-hr.-old culture (in the lag phase of growth) is most resistant to both ultra-violet radiation and desiccation, whereas the 4·5-hr.-old culture (in the logarithmic phase of growth) is least resistant to these agents. The 24-hr-old culture (in the maximum stationary phase) is fairly resistant to both the ultra-violet light and desiccation.

The resistance of *E. coli* to ultra-violet light and desiccation seems to be a maximum in the lag phase and starts diminishing during early logarithmic phase and is regained as the culture reaches the maximum stationary phase. The resistance to desiccation seems to be completely regained after 24 hr., but the resistance to ultra-violet radiation is not completely regained even after 24 hr.

It has been observed (ref. 4) that during the later part of the lag phase the nucleic acid content, protein content and the cell size are at their maximum levels and start diminishing in logarithmic phase. It is also well known that the bacterial cells are not dividing in the lag phase and their cell walls and membranes are unbroken. It therefore appears that the intact cell membranes, the large amount of nucleic acids, etc., and the large cell size may be responsible for the resistance of the cells in this phase to ultra-violet radiation and desiccation.

It is interesting to note that work conducted on the effect of X-rays, heating, cooling, freezing and freeze-drying, seems to suggest that bacteria are less resistant to these agents in the logarithmic phase than in the stationary phase. It would be extremely informative to investigate the effects of these agents on the bacteria in the lag phase.

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Spheroplasts of Marine Bacteria induced by the Action of Penicillin

Several species of marine bacteria which are characterized by osmotic dependence have been induced to form spheroplasts by the action of penicillin added to cultures growing optimally in a nutrient broth prepared with sea water. Two factors indicated the likelihood of obtaining this result. First, recent papers[1-3] have contained results indicating that the internal osmotic pressure of marine bacteria and

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halophiles was equal to, or less than, the external environment; consequently, spheroplasts of these bacteria should be stable in the growth medium. Second, the media, in particular those containing sea water, are characterized not only by a high osmotic pressure but also by a high content of magnesium ions, a characteristic of solutions used to stabilize penicillin-induced spheroplasts of Gram-negative bacteria[4]. The techniques for inducing spheroplasts of marine bacteria and a comparison of the osmotic fragilities of cells and spheroplasts of one species are described briefly in this communication.

The five types of bacteria used have been described recently[5]; all were Gram-negative rods characterized by osmotic fragility and salt requirements for growth. The medium consisted of a tryptic digest of casein, trypticase (Baltimore Biological Laboratory, Inc.), 1 per cent, dissolved in artificial sea water of the following composition: sodium chloride, 0·4 *M*; magnesium chloride, 0·05 *M*; sodium sulphate, 0·01 *M*; and potassium chloride, 0·01 *M*. The experimental cultures were grown in 20-ml. amounts of medium in 250-ml. Erlenmeyer flasks each fitted with a side-arm tube to allow periodic measurement of turbidity. Subsequent to inoculation, as increasing turbidity indicated growth, penicillin *G* was added to the flasks. Three amounts of penicillin, to give final concentrations of 1·0, 100 and 1,000 units per ml., were tested. Wet mounts of cultures were prepared hourly and examined by phase microscopy.

The osmotic fragilities of cells and spheroplasts of one species (*MB* 22) were compared by determining the turbidity[6] and indole formation[7] of preparations washed in sea water and suspended in graded concentrations of sodium chloride. The turbidity was measured photometrically with a Spectronic 20. The indole formed from L-tryptophan was determined by the method of Wood, Gunsalus and Umbreit[8].

Morphological alteration was observed in all species after 1 hr. cultivation in the medium with 100 units of penicillin per ml.; after 2 hr. all cells of four of the five species had been converted to spheroplasts. The sequence of spheroplast formation was typical[4], from the initial 'rabbit ear' structure to the formation of spherical bodies having the crescent-shaped refractile area. One organism, *MB* 2, did not form spheroplasts, but showed greatly enlarged and swollen 'rabbit ear' structures. After 5 hr. in 1·0 unit of penicillin per ml., *MB* 14 had formed spheroplasts, *MB* 22 had formed serpentine cells about ten times normal length, and the other species showed no apparent change. One organism (*MB* 21), a peritrichous Gram-negative rod, was converted to spheres after 2 hr. incubation with 100 units per ml. of penicillin, but examination after 5 hr. showed the culture to have returned to its rod form, possibly by virtue of an active penicillinase. Motility persisted in the cultures treated with penicillin, in some cases even after the formation of spheres, but was lost in all species, with the exception of *MB* 21, after several hours.

Table 1. Comparison of Osmotic Fragilities of Cells and Spheroplasts of *MB* 22

Molarity of sodium chloride in suspending fluid	Residual turbidity (per cent)*		Residual indole formation (per cent)†	
	Cells	Spheroplasts	Cells	Spheroplasts
0·0	73	17	0	0
0·1	86	83	60	40
0·2	95	95	93	47
0·3	95	95	100	93
Artificial sea water	100	100	100	100

* Percentage of optical density as compared with optical density in artificial sea water. Optical density of cells in artificial sea water, 0·56; optical density of spheroplasts in artificial sea water, 0·59.

† Percentage of indole formed as compared to indole produced in artificial sea water (10·6 μgm./10 min.).

Comparison of the osmotic fragilities of cells and spheroplasts of *MB* 22 (Table 1) indicates that tryptophanase activity and turbidity are both dependent on the osmotic pressure of the fluid in which the cells are suspended. However, it cannot be said with certainty that the spheroplasts are more susceptible to lysis than the cells. The low residual turbidity of the spheroplasts in distilled water may indicate the lack of cell wall material. Microscopic observation of the distilled water lysates of spheroplasts showed only disorganized debris while cell wall ghosts were seen in similar lysates of the cells.

The formation of stable spheroplasts from marine bacteria in a culture medium having an osmotic pressure equivalent to that of sea water suggests that the marine bacterium may not require the rigid cell wall to maintain its cellular integrity in the natural environment. Possibly the rigid cell wall indicates the evolution of these forms from organisms adapted to more hypotonic environments, since it is in the latter circumstances that the rigid cell wall of bacteria has a selective advantage making the organism osmotically independent of its environment.

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Flagellar Changes in *Escherichia coli* induced by Temperature of the Environment

The effect of change of environmental temperature on the motility of a strain of *Escherichia coli*, type I (N.C.T.C. 8196) has already been described[1]. This strain, which appeared to be non-motile when examined after 18-hr. growth at 37° C. in meat extract broth or in the same medium solidified with agar, was found to be motile in the early stages of exponential growth (5 hr. at 37° C.) and after growth for 18 hr. or longer at 20° C. Storage of non-motile cells grown at 37° C. at a lower temperature (20° C.) for 1–2 days led to the recovery of motility by most of the cells in the culture. Cells grown at 20° C. retained motility for several weeks, but lost it rapidly when transferred to the incubator at 37° C.

The effect of temperature of growth and storage on a further fifteen strains of *E. coli* has since been investigated, and seven of these showed rapid loss of motility in cells grown at 37° C. and long-lasting motility in cells grown at 20° C. Of the remaining eight strains some were non-motile at all growth temperatures and others developed and retained motility at growth temperatures of 15–37° C. In these seven strains, showing transient motility when grown at 37° C. and long-lasting motility at lower growth temperatures, there was some variation in the recovery of motility when non-motile, flagellate cells

Table 1. Effect of Growth Temperature on Flagella, Motility and Cell Yield in Cultures of Three Strains of *E. coli* Type 1 Grown for 24 hr. in Meat Extract Broth

Growth temperature (° C.)	Strain *W.I.G.*			Strain *J*			Strain 1093		
	Cell No. (percentage of light absorbed)	Flagella (percentage of cells flagellate)	Motility (percentage of cells motile)	Cell No. (percentage of light absorbed)	Flagella (percentage of cells flagellate)	Motility (percentage of cells motile)	Cell No. (percentage of light absorbed)	Flagella (percentage of cells flagellate)	Motility (percentage of cells motile)
15	13	65	1–5	32	0	0	30	50	40–50
20	28	85	80–90	38	65	60–70	41	100	20–30
23	48	90	< 1	60	65	60–70	72	75	5–10
25	67	95	0	68	65	60–70	77	75	1–5
30	79*	85	0	79*	55	10–20	80*	75	< 1
36	77	60	0	78	25	0	74	40	0
40	41	25	0	68	< 1	0	65	30	0
44	29	0	0	49	0	0	53	0	0

* Optimum temperature for cell growth.

were stored at 20° C. The recovery of motility varied from a minimum of 1–5 per cent of the cell population to a maximum of about 80 per cent, according to the strain. This recovery of motility at lower temperatures could not be wholly accounted for by the growth of new cells, since in some strains the number of cells with recovered motility greatly exceeded the increase of population. It should be emphasized that the loss of motility at 37° C. was not, in most cases, due to a loss of flagella since in six of these seven strains cells grown or stored at 37° C. retained their flagella. The flagella had lost the power to propel the cell—they had become paralysed.

The influence of temperature on motility in a number of bacterial species has been described[2–5], but in the examples reported loss of motility would seem to be due to loss of flagella in cells grown at a high temperature or loss of function in already-formed flagella in cells exposed to a high temperature. Thus the rapid loss of function by flagella during growth at the optimal temperature (30–37° C.) and the prolonged maintenance of function by flagella formed at lower temperatures seemed to merit further investigation.

As mentioned earlier, a total of sixteen strains of *E. coli* type I were examined. They consisted of four laboratory stock cultures and twelve strains of *E. coli* type I consecutively isolated in the bacteriological laboratory of the Western Infirmary, Glasgow. They possessed the following characters: lactose fermented with acid and gas, indole formed, methyl red test positive, Voges–Proskauer test negative and no growth in Koser's citrate. Motility was determined in wet films from meat extract broth cultures or from the condensate water of meat extract agar slopes. Rough estimates were made of the proportion of motile cells, the estimates being checked by a second observer. Flagella were demonstrated and counted in films stained by Leifson's stain[6] or Kirkpatrick's silver stain[7].

Out of the seven strains showing motility after 5-hr. growth at 30–37° C. and loss of motility after 18-hr. growth at this temperature, the first three examined were subjected to more detailed investigation. Each strain was grown in meat extract broth at temperatures ranging from 15 to 44° C. At the end of 24-hr. incubation the proportion of cells showing flagella and the proportion of motile cells were determined. The amount of growth was measured turbidimetrically in the Spekker absorptiometer (previous measurements showed that the percentage of light absorbed was closely related to cell numbers). The results are shown in Table 1. The finding that the optimum temperature for demonstration of motility in 24-hr. cultures may be considerably lower than the optimum temperature for cell yield confirms what most bacteriologists find. The three strains show an essentially similar pattern although they differ in regard to the optimum temperature for growth, flagella, and motility. Thus it is possible to distinguish three types of cell in each strain: (*a*) non-motile, non-flagellate cells grown at elevated temperatures (above 40° C. for strain *W.I.G.*); (*b*) non-motile, flagellate cells grown over a middle range of temperatures (23–40° C. for strain *W.I.G.*); (*c*) motile, flagellate cells grown at low temperatures (below 23° C. for strain *W.I.G.*). Another way of putting it would be to say that in the upper range of growth temperatures flagella are not produced; in the middle range they are formed, but lose activity rapidly—they become paralysed; and in the lower range flagella are formed which retain their function for 24 hr. or more (strain *W.I.G.* is still motile after storage for 3 weeks at 20° C.).

An inevitable question is whether there are any differences in structure or composition between active and paralysed flagella; and the simplest approach, perhaps, is to look for antigenic differences. This was done and differences were found. Rabbits were immunized with formolized cells of the three types of strain *W.I.G.*—non-flagellate cells grown at 43° C.; flagellate, non-motile cells grown at 36° C.; and flagellate, motile cells grown at 20° C. The resulting antisera showed, as one might expect, considerable cross-reactions. But, after a number of absorptions with cells heterologous in regard to growth temperature, sera specific for the homologous cells were obtained. Table 2 shows the results of experiments in which absorbed antiserum prepared against cells grown at 36° C. (flagellate, non-motile) and absorbed antiserum prepared against cells grown at 20° C. (flagellate and motile) were titrated against formolized '*H*' suspensions of the three types of cell listed here. That these results are due to the presence of antibodies to specific flagellar antigens is shown by our finding that the absorbed antisera did not agglutinate '*O*' suspensions or cells which had been stripped of their flagella by high-speed agitation in the M.S.E. blender[8]. Clearly, therefore, there are antigenic differences between paralysed flagella produced by growth at elevated temperatures and active flagella formed in growth at lower temperatures.

Table 2. Agglutination of Cells of Strain *W.I.G.* grown at 43° C., 36° C. and 20° C. against Antisera prepared against Cells grown at 36° C. and 20° C. and later absorbed with Non-homologous Cells

Agglutination tubes incubated for 4 hr. at 56° C. and 20 hr. at 20° C.

Cell suspension of cells grown at temperature shown (° C.)	Agglutination titre as reciprocal of dilution of antiserum	
	Antiserum to 36° C. cells (absorbed by cells grown at 20° C. and 43° C.)	Antiserum to 20° C. cells (absorbed by cells grown at 36° C. and 43° C.)
43	Nil	Nil
36	1,280	Nil
20	Nil	640

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Summarizing, one might say that in the sixteen strains of *E. coli* type I examined, seven showed the possible existence of two forms of flagella—a functional or active form produced in growth at low temperatures and a non-functional or paralysed form produced by growth at higher temperatures. Conversion from one form to the other could be obtained in varying degree by switching the incubation temperature. For the one strain examined serologically, these two forms differed antigenically.

No simple explanation of the phenomenon occurs to us. One can scarcely account for it by postulating exhaustion of nutrients or accumulation of toxic metabolites at the higher temperature, since cells which had lost motility could regain it on transfer to a lower temperature. In some strains the number of cells recovering motility exceeds the number of new cells formed. Loss of motility through oxygen deficiency would seem to be ruled out by the occurrence of the phenomenon in actively aerated cultures. Nor is it enough to say that high temperatures favour a flagellar structure inimical to motility since young cells are motile at elevated temperatures. One might, of course, postulate the accumulation of toxic products at higher temperatures which are subjected to further change in reactions favoured by lower temperatures, but this ignores the finding of antigenic differences. A satisfactory explanation still eludes us.

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Free-Radical Formation during Storage of Freeze-dried *Serratia marcescens*

Bacteria which have survived freeze-drying retain the capacity to regenerate colonies after prolonged storage under vacuum, in contrast to their rapid loss of this capacity when stored in air. The question of whether bacteria stored under such conditions are dormant or whether some metabolic activity continues to maintain the bacilli in a living state is of fundamental importance to a further understanding of the physiology of life in low-moisture environments. Heckly[1] has reviewed many of the problems associated with freeze-drying. Results of preliminary work on freeze-dried cells had indicated a slow gaseous uptake when the material was stored in dry air and a greater uptake if stored under moistened air. Because the results were not reproducible, other techniques were examined, specifically that of electron paramagnetic resonance, in the hope of finding an indirect measurement of respiration. The fundamental theory and practice of electron paramagnetic resonance has been reviewed by Ingram[2], while a review of biological applications of the phenomenon has been given by Sands[3].

Serratia marcescens was grown in Bunting's[4] medium (modified, except in one instance, by withholding the iron citrate) in shake flasks at 34° C. for 24 hr. Cells were collected by centrifuging and resuspended in 0·066 *M*, *p*H 7·0, potassium phosphate buffer by mixing with a pipette for about 5 min. The suspension was recentrifuged and again the cells were suspended in buffer fortified, except in one instance, by the addition of lactose[5]. Suspensions were divided into two portions; one portion was either placed in an autoclave or treated with mercuric chloride to kill the bacilli; the other portion was untreated.

Preparations were distributed, 16 ml. per 60 ml. Army-style vaccine bottle, shell frozen, and the bottles were placed under vacuum (ambient temperature, 24 hr., 2–5μ mercury pressure). All preparations in a given experiment were frozen and dried simultaneously. The bottles were then removed to a dry-box and the dried material was transferred to either weighed glass vials or into quartz tubes suitable for electron paramagnetic resonance work. Vials and tubes were then flame-sealed either under atmospheric pressure or at a pressure of less than 10μ mercury. In one instance the air was replaced with water-pumped nitrogen. Reconstitution of the dried material was accomplished by adding an appropriate volume of sterile distilled water to the bottles. Viable cell numbers were determined by serial decimal dilution followed by surface colony counts on Bunting agar plates. Colonies were counted after 24 hr. incubation of the plates. A Varian *V*–4500 electron paramagnetic resonance spectrometer with 100-kc./s. field modulation and a 6-in. magnet were used to obtain the spectra of the dried samples.

Viable dried bacilli were found to exhibit a weak, single-line resonance spectrum having a *g*-value near 2, and of line-width approximately 11 gauss (peak-to-peak on derivative curves). A similar phenomenon has been reported for other dried biological samples[6]. Samples sealed in air, however, showed a marked increase in the intensity of their resonance spectra with time. A sample stored 90 days under vacuum produced no augmented signal, but the signal immediately showed a change in intensity when the vial was opened to the atmosphere.

In Fig. 1 the reciprocal of the relative intensity, as the fraction of the original signal intensity, is plotted against time and the loss of colony-forming units is plotted on the same scale. The results show that the most rapid increase of the signal occurred during the first week of storage, after which the rate decreased. The concentration of radicals in these samples, determined by comparison with a standard sample of known concentration, was approximately 10^{12}/mgm. of sample, whereas the initial number of viable bacilli varied between 4 and 8 $\times$ 10^{9}/mgm.; there were about 200 radicals formed per living bacillus. An outline of experimental details and results is shown in Table 1.

The vial associated with experiment 1 *b* (Table 1) was re-evacuated overnight after a fairly high number of radicals had formed, but the signal did not decrease. The vial was then opened and distilled water was added to the contents. Subsequent analysis failed to show the presence of a measurable free-radical content.

These results may be interpreted in three ways: (a) An easily oxidized molecule, perhaps a ring structure, may have reacted, non-enzymatically, with oxygen to form a free-radical which was stable under the low moisture conditions. Scholes *et al.*[7] have discussed reactions of this nature. Such substances could cause the death of a cell at the moment of

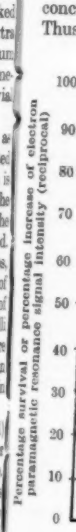

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Table 1. SUMMARY OF THE PREPARATION AND STORAGE OF FREEZE-DRIED *Serratia marcescens* AND THE ELECTRON PARAMAGNETIC RESONANCE SIGNALS NOTED THEREAFTER

Exp. No.	Variation of storage medium	Pre-treatment	Storage atmosphere	Percentage of immediate recovery	Electron paramagnetic resonance spectra
1*a*	Iron + lactose	autoclaved	air	zero	constant medium strength
b	Iron + lactose	none	vacuum	100	weak*
c	Iron + lactose	none	air	100	small, changing to large
2*a*	No iron, no lactose	$HgCl_2$	air	zero	weak
b	No iron, no lactose	none	vacuum	2·0	weak
c	No iron, no lactose	none	air	2·0	weak, but slightly increased after 40 days
3*a*	No iron, lactose	none	vacuum	100	weak
b	No iron, lactose	none	air	100	small, changing to large
4*a*	No iron, lactose	none	nitrogen	80	faint, no appreciable immediate change
b	No iron, lactose	none	air	80	small, changing to large

* The vial was opened to the atmosphere after 6 weeks and re-sealed. A signal then appeared.

reconstitution. (*b*) An enzyme system may have been injured or inhibited, resulting in the accumulation of a free-radical intermediate within the cell. Presumably, the injury might have caused the death of the cell, though not necessarily, because the accumulated radical could act as suggested in (*a*). In support of this suggestion, Yamazaki *et al.*[6] have shown unequivocally that radical intermediates are formed in typical enzymatic oxidations. (*c*) The relatively low number of radicals per bacillus indicates that a combined form of a transition element present in trace amounts could have been responsible for the signal analogous to (*a*).

A strong similarity appears to exist between the loss of viability and the growth of the free-radical concentration in the sense that bacilli stored under vacuum conditions, which favour high retention of viability, showed no change in radical concentration, whereas those stored under atmospheric conditions, an adverse environment, exhibited a consistent rapid increase of free-radical concentration. Furthermore, a marked change occurred in both the rate of loss of viability and the rate of increase of the radical concentration after about one week's storage (Fig. 1). Thus, a correlation is indicated between the death process and the growth of the free-radical concentration, although present results preclude the assignment of a direct cause-and-effect relationship.

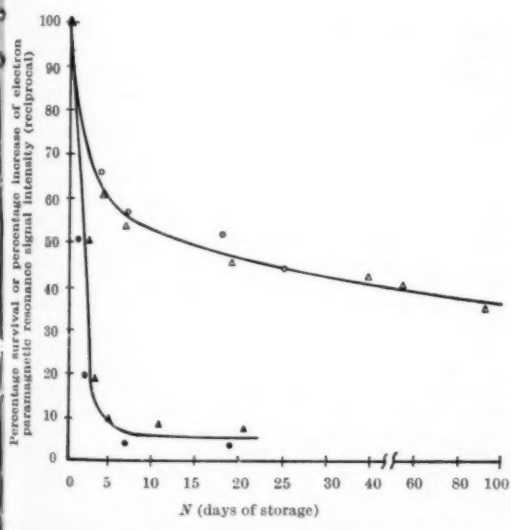

Fig. 1. Comparison of the change in electron paramagnetic resonance signal intensity with the loss of viability of *Serratia marcescens* stored in air. The points ○ and ● are for a single preparation; △ and ▲ are from different preparations.

●▲, percentage survival, $100 \times \frac{\text{No. of viable cells at } N \text{ day}}{\text{No. of viable cells at 0 day}}$.

○△, percentage electron paramagnetic resonance intensity increase, $100 \times \frac{\text{intensity at 0 day}}{\text{intensity at } N \text{ day}}$

Both oxygen and moisture are involved in the loss of colony-forming units. Monk *et al.*[9] have shown that viability is lost most rapidly when the bacteria contain about 30 per cent moisture. Definitive data concerning this point might be forthcoming if one compared the production of free radicals to the loss of viability under varied moisture conditions, or determined whether a reduction of the free-radical content increased the apparent number of colony-forming units. The evidence suggests that some changes, perhaps metabolic in nature, occur in the dried state.

This work was sponsored by the Office of Naval Research under a contract with the University of California.

After this communication was submitted, our attention was directed to the finding by Lion *et al.*[10] that free radicals are formed during storage of freeze-dried *Escherichia coli*. This, together with our subsequent finding that similar phenomena occur when freeze-dried *Sarcina lutea* or *Micrococcus radiodurans* are tested, indicates that free-radical formation associated with the death of dried micro-organisms is generalized in nature. We take exception, however, to the suggestion that normal metabolic reactions might not be involved, because the evidence points strongly toward the oxygen-transport system as being that portion of the metabolic make-up of living structures most likely unbalanced by oxygen under dry conditions.

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VIROLOGY

Serial Propagation of Types 1, 2, 3 and 4 Dengue Virus in HeLa Cells with Concomitant Cytopathic Effect

Attempts to obtain cellular destruction with dengue in stable human cell lines have thus far been unsuccessful. Banta[1] was unable to produce a specific cytopathic effect with type 1 dengue virus in HeLa cells. Beasley *et al.*[2] demonstrated replication of type 2 dengue virus on primary infection of *KB* cells, but concomitant cell damage was not observed.

It has been pointed out by Dubos and Schaedler[3], and is indeed an established clinical fact, that virus diseases often run a milder course in malnourished than in well-fed persons. There is evidence also that *in vitro* processes necessary for viral multiplication are influenced by the state of health of the cell cultures. Thus, Morgan *et al.*[4] have shown that in tissue culture, viruses remain dormant so long as the infected cells are maintained in deficient culture media, while Enders[5] has emphasized that any apparent effect of a given substance on viral multiplication may be quite indirect and related only to preservation of cell viability. Cell viability, especially in work carried out with stable cell lines, depends both on nutrients available during the growth period and on substances used in the maintenance medium following infection of cells. The latter conditions have been studied more extensively. Thus, Ginsberg *et al.*[6] introduced tryptose phosphate broth (Difco) as a supplementary beneficial factor for maintenance of HeLa cells.

I, conversely, have been interested in variation of the growth-promoting medium and the effects of this variation on subsequent viral synthesis, in particular on induction of a clear-cut, reproducible cytopathic effect. During the past five years, a standard HeLa (Gey) cell line, obtained in 1954 from the Tuskegee Institute, Alabama, has undergone more than 335 regular transfers in a growth-promoting medium containing among other ingredients 15 per cent tryptose phosphate broth. Monolayers of HeLa cells cultivated over long periods in this medium are less easily broken up by 0·25 per cent trypsin (Difco) than are those of HeLa cells grown conventionally in adult human serum and Eagle's basal medium. It is assumed, although experiments pertaining to this question have not yet been terminated conclusively, that this difference is due primarily to the ability of tryptose phosphate broth to produce a high degree of cohesion between the individual cells. Whereas non-infected HeLa tryptose phosphate broth cells show good cell viability during experimental stress periods and do not become detached easily from the tube wall, infected HeLa tryptose phosphate broth cells round up and become detached readily from glass, thus exhibiting a clear-cut cytopathic effect which is easily distinguishable at an early stage. The HeLa tryptose phosphate broth cell line has been tested experimentally since 1957 and has responded with regular cytopathic effect to the action of approximately 40 arbor viruses[7–9], including St. Louis encephalitis and Japanese *B* encephalitis, both of which gave irregular results in the hands of Scherer and Syverton[10].

In the present work, HeLa tryptose phosphate broth cells, grown in slant-type tubes in 30 per cent human serum, 10 per cent fœtal bovine serum, 45 per cent Hanks's balanced salt solution and 15 per cent tryptose phosphate broth, were washed once with Hanks's balanced salt solution, and were then inoculated with ten-fold dilutions of 10 per cent suckling mouse brain suspensions of each dengue virus: type 1 (ref. 11), Hawaiian strain, seventy-eighth passage; type 2 (ref. 12), New Guinea strain, forty-fifth mouse passage; type 3 (ref. 13), twenty-sixth mouse passage; and type 4 (ref. 13), twelfth mouse passage. Cell cultures were maintained in 97 per cent Eagle's medium and 3 per cent fœtal bovine serum, and were examined at intervals for 21 days. Careful attention was given to the *p*H of inoculated cultures, which was regulated at $7{\cdot}2 \pm 0{\cdot}2$. Fluids were changed daily during the first week following cellular infection and thereafter three times weekly.

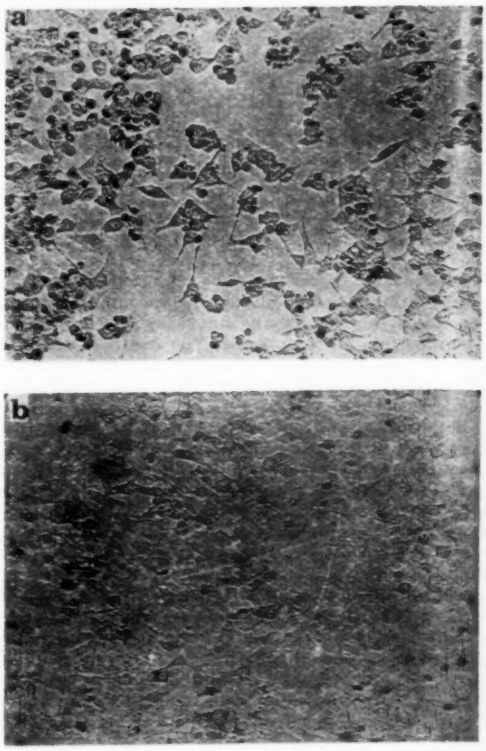

Fig. 1. Unstained HeLa cell cultures in slant-type tubes inoculated with (*a*) normal rabbit serum and type 4 dengue virus, (*b*) with anti-type 4 rabbit serum and type 4 dengue virus. (× *c.* 133)

The results (Table 1) show that the four immunologically different types of dengue virus can be propagated with concomitant cytopathic effect in HeLa tryptose phosphate broth cells. During serial tissue culture passages, carried out at 7-, 10- or 14-day intervals, TCD_{50} titres per 1 ml. ranged from 10^{-3}–10^{-6} for type 1, 10^{-4}–$10^{-6.5}$ for type 2, 10^{-2}–$10^{-4.0}$ for type 3 and $10^{-2.5}$–10^{-5} for type 4. The cytopathic effect was similar for all virus types (Fig. 1*a*) except that it was induced more rapidly by types 2 and 3. Five to seven days following inoculation of 1,000–10,000 TCD_{50}, focal lesions made up of rounded and granulated cells appeared throughout the cell sheet. These micro-plaques became more numerous and confluent during the second week post-inoculation. During the third week 50–70 per cent

Table 1. SERIAL PASSAGE OF TYPES 1, 2, 3 AND 4 DENGUE VIRUS IN HeLa TRYPTOSE PHOSPHATE BROTH CELLS

Virus type	No. of *in vitro* passages	TCD_{50} (neg. $\log_{10}$ per 1 ml.)	Cumulative $\log_{10}$ of dilution of original inoculum
1	1	3·0	1
	7	6·0	15
	11	4·0	20
	14	5·5	27
	21	4·8	41
2	1	5·5	3
	6	4·0	15
	9	6·5	22
	14	5·0	44
	27	6·5	75
3	1	5·5	5
	3	3·0	7
	7	5·5	15
	10	4·5	27
	18	6·0	53
4	1	4·5	1
	2	2·5	2
	5	4·5	11
	8	5·0	19
	16	4·0	37

of the cells had become detached from glass and disappeared into the maintenance medium, and the cells still attached to the tube wall were rounded and granulated. Only a few healthy cells, from which regeneration occurred during observation of cell cultures during 1–2 months, were present. When inoculated into 2-day-old mice by the intracerebral route, fluids of cultures exhibiting cytopathic effect proved fatal within 6–10 days. The development of cytopathic effect, similar to that described by Hotta and Evans[14,15] for types 1 and 2 dengue virus propagated in monkey kidney cell cultures, was inhibited by type-specific immune serum (Fig. 1*b*). In the *in vitro* neutralization tests, undiluted, freshly drawn[16] serum samples from immunized and normal rabbits were used.

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Reproduction of Tick-borne Encephalitis Virus in HeLa Cells

To examine the exact mechanism of reproduction of the tick-borne encephalitis virus in susceptible host cells *in vitro*, the intracellular proliferation and release of the virus into the medium have been compared with the sequence of morphological changes studied by different methods. HeLa cell monolayer cultures were used, infected with a large or a small dose of a selected variant of the Czechoslovak strain *Hypr* of tick-borne encephalitis virus[1], which is cytopathic for HeLa cells.

To ensure that materials were prepared under identical conditions for all the methods used, the contents of one Roux flask were collected for each time period, and infected cells were obtained from a homogeneous cell sheet, covering the bottom and cover-slips stuck to it by chicken plasma. Results obtained by the following methods were compared: (i) virus assessment in the cultural fluid and in cell crushes by repeated freezing and thawing; (ii) intracellular localization of virus by the fluorescent antibody method, using high-titre anti-*Hypr* horse γ-globulin conjugated with fluorescein isothiocyanate[2]; (iii) phase-contrast microscopy of living cells for cytopathic changes.

After an infection with 3,200 LD_{50} per cell, virus proliferation began between the eighth and eleventh hour after infection and reached its maximum in 24 hr. (Fig. 1). Corresponding with these results, specific fluorescence, indicating intracellular virus

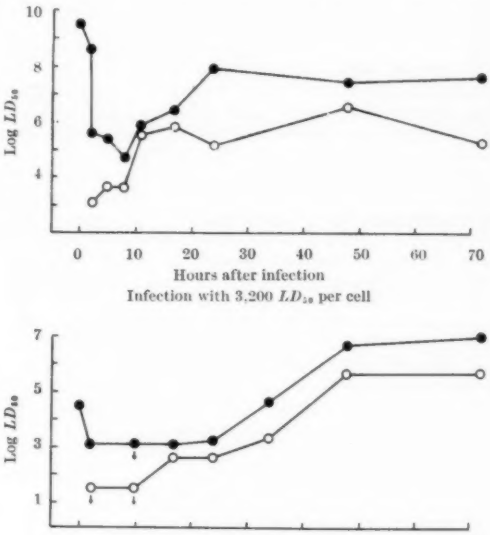

Fig. 1. Reproduction of a selected cytopathic variant of tick-borne encephalitis virus (strain *Hypr*) in HeLa cells. ●, Free virus in the whole medium (40 ml.); ○, total intracellular virus

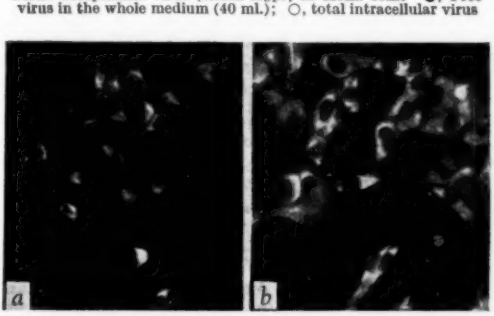

Fig. 2. HeLa cells 12 hr. (*a*) and 24 hr. (*b*) after infection with tick-borne encephalitis virus. Virus localization by fluorescent antibodies

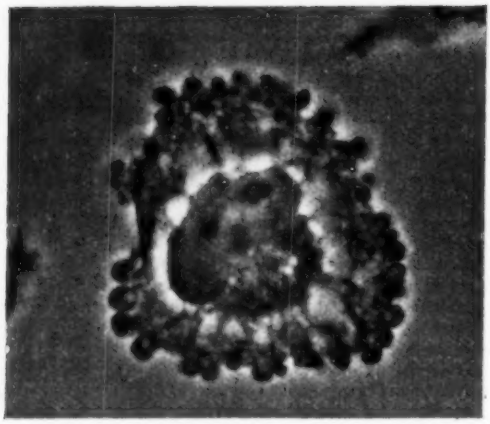

Fig. 3. Vacuolization and protrusions on cell surface of HeLa cell 24 hr. after infection with tick-borne encephalitis virus

material, was present in the majority of cells beginning with the eleventh hour after infection and intensified up to the twenty-fourth hour, starting in the paranuclear area (Fig. 2*a*) and extending later to the rest of the cytoplasm (Fig. 2*b*). At the end of the twenty-four hour period, well-marked cytopathic changes appeared in some cells, with vacuolization and protrusions on the cell surface (Fig. 3).

An infection with a small dose of virus (1 LD_{50} per 33 cells) led to the first slight increase of intracellular virus between the tenth and seventeenth hour, followed by a further increase which started with the twenty-fourth hour and reached a maximum 48 hr. after infection. The first fluorescence was rather delayed in appearance and was a maximum in not more than 50 per cent of the cells of the tissue culture. The initial cytopathic changes appeared approximately at the same time as the fluorescence.

Reproduction of other viruses of the tick-borne encephalitis complex in different cell cultures by these methods is under investigation.

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Accumulation of Asparagine in Maize Plants infected by Maize Rough Dwarf Virus and its Significance in Plant Virology

It has already been shown that the rough dwarf disease of maize, so far known only from Italy, Spain and Israel, is caused by a virus[1]. Since this virus has a pronounced dwarfing effect on maize seedlings, which in most cases proves lethal, it would be expected that certain physiological abnormalities could be determined at a rather early stage of infection. The effect of this virus on the balance of free amino-acids in infected maize plants is being studied in our laboratory. During the course of this work an abnormal accumulation of asparagine was noticed in all plants bearing the virus infection symptoms, whereas in all healthy plants of the same age this amide was at the most only faintly detectable by the method of two-dimensional paper chromatography which we used[2]. Moreover, when *l*-asparagine was added to the sap extracted from diseased plants and chromatographed, its spot coincided with the one suspected to be that amide.

Later, a quick method was worked out for determining the presence of asparagine in sap of plants infected by this virus. The test is based on the distinctive orange-brown colour which asparagine gives with ninhydrin[3]. Fresh maize stalks were ground in a meat mincer; the sap squeezed through cheese cloth and then centrifuged for 10 min. at 4,000 r.p.m. For the ninhydrin test 1 ml. of the supernatant was heated for 3 min. in a boiling water-bath, diluted 10-fold in water and then filtered through Whatman No. 1 filter paper. The reaction mixture includes 0·3 ml. diluted sap solution and 1 ml. of 0·25 per cent of ninhydrin in absolute ethanol. Colour was developed by boiling in a water-bath for 2 min. followed immediately by adding 2 drops of sulphuric acid (1 : 6) and agitating the test-tube. The blue coloration disappeared in a very short time and the orange-brown coloration formed by asparagine turned yellow. The latter colour is sufficiently stable as to allow a qualitative test, although it, too, fades away slowly and therefore cannot serve as a quantitative method. It should, however, be mentioned that proline and hydroxyproline give a different heat-stable coloration on boiling with ninhydrin, but these two amino-acids did not show up on our chromatograms and therefore do not interfere in this case.

It should also be pointed out that the relative amount of sap obtained from diseased plants was noticeably higher than that from non-infected ones. This rules out the possibility of attributing the high asparagine content merely to an alleged lower water content of infected plants.

The ninhydrin test of sap was always strongly positive for all maize seedlings showing rough dwarf symptoms, whereas for healthy plants it was negative, or very seldom, weakly positive. The positive reaction, however, should not be attributed wholly to asparagine, since glycine, tyrosine, phenylalanine, histidine and tryptophan all give an orange-brown coloration with ninhydrin[3]. Part of the yellow colour obtained may perhaps be due to these and other reactions; but it is mainly due to asparagine, as was shown on our chromatograms.

The changes in free amino-acids and amides of the host plant resulting from virus infection were recently reviewed by Porter[4], who mentions at least three instances where virus infections in plants were followed by a net increase in asparagine, as compared with healthy plants. Further evidence is now available concerning at least eight more virus diseases, in which specifically asparagine is reported to accumulate in the host plant following infection. These are tobacco leaf curl virus in *Nicotiana* leaves[5], papaw mosaic and papaw leaf curl viruses in papaw leaves[6], *Ricinus* mosaic virus in castor bean leaves[7], *Luffa* yellow mosaic virus in *Luffa* leaves[7], potato spindle tuber virus[8] and potato leaf roll virus[9] in potato tubers, and finally the present record on maize rough dwarf virus. The only contradicting evidence is by Commoner and Nehari[10], who observed a deficiency in asparagine content in disks of tobacco leaves infected with tobacco mosaic virus. Since these measurements were made on leaf disks, even though floated in

nutrient solution, their results cannot be safely applied to the situation in the whole plant. In fact, when the same experiments were repeated on intact plants[4], a net increase in asparagine content was evident as early as during the first 120 hr. following infection.

Asparagine, as well as glutamine, are known to accumulate in tissues of plants growing under unfavourable conditions, whereby proteolysis and subsequent deamination bring about an increased release of ammonia. The presence of these compounds in higher concentrations in virus-infected plants should not be regarded as merely resulting from some secondary effects on the protein metabolism of the diseased plant. It has already been indicated[11] that these amides are implicated—whether directly or indirectly—in the biosynthesis of purine, pyrimidine, nucleosides and nucleotides, thus bearing great significance in relation to nucleic acid metabolism, leading to the biosynthesis of the virus material itself[12]. This may also offer some explanation for the specificity of the occurrence of asparagine in tissues of plants infected by viruses and for its conspicuous rareness in cases of plant diseases caused by other pathogens.

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GENETICS

Radiosensitive Stages in the Spermatogenesis of *Drosophila melanogaster*

The existence of differential radiosensitivity during certain stages of spermatogenesis in *Drosophila melanogaster* was first proposed by Lüning[1]. Further evidence provided by Auerbach's[2] three-day brood technique suggested that the highest mutation frequency observed in the 3–6-day period following treatment indicated peak sensitivity in immediately post-meiotic stages. Additional support for this view that spermatids were particularly susceptible to the mutagenic action of X-rays was derived principally from the results of irradiating immature sperm in the pupal testis[3,4]. More definitive daily brood analysis[5], however, established a marked dominant lethal frequency ranging over the sixth, seventh and eighth days following the irradiation of newly emerged males, but from these results Bateman was able to draw no firm conclusion other than that this probably represented sensitive meiotic and post-meiotic stages. Using the same technique, Ives[6] ascribed the first appearance of induced cross-overs on the seventh day after irradiation to the utilization in this brood of

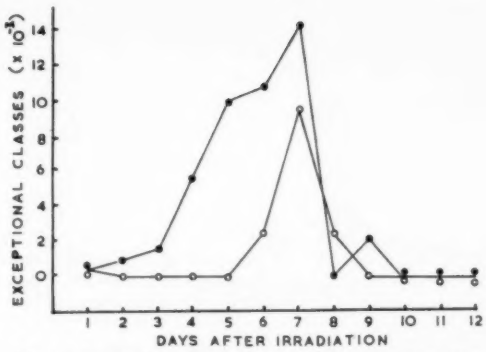

Fig. 1. Brood patterns for the 12 days following irradiation. ○, *XXY* females produced by non-disjunction in immature sperm; ●, *X0* males produced by loss of *X* or *Y* chromosome

sperm treated in early meiotic and pre-meiotic stages. In a similar experiment, he obtained a maximum frequency of both autosomal recessive and sex-linked recessive lethal mutations on this same day. Sävhagen[7] considered sex chromosome loss and non-disjunction also in parallel investigations. Here again, the highest frequencies of *X0* males were observed on those days which, by comparison with the non-disjunction experiment, probably corresponded to stages treated before anaphase I of meiosis.

This communication is a preliminary account of experiments designed to detect simultaneously complete loss of *X* or *Y* chromosomes, deletion in the *Y* chromosome and non-disjunction between *X* and *Y* chromosomes. This is achieved by means of a new, doubly marked *Y* chromosome stock kindly provided by Dr. D. L. Lindsley of Oak Ridge. This *Y* chromosome, designated $sc^8\ YB^s$, has the normal allele of the *X* chromosome marker yellow (y^+) inserted into the short arm and the dominant Bar-Stone marker (B^s) into the long arm. After X-irradiation with 1,000 r., males with a wild-type *X* chromosome and this doubly marked *Y* are mated daily to three females homozygous for the *X* chromosome markers yellow, apricot, echinus (*y apr ec*). The normal offspring from this

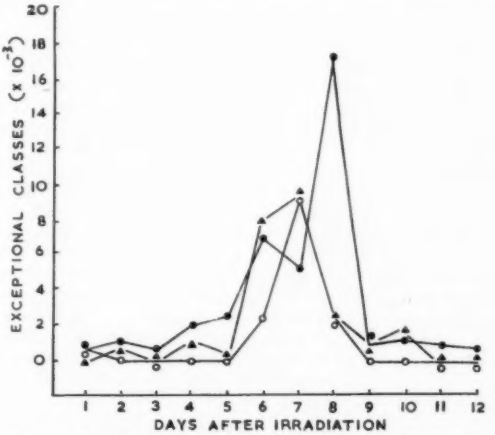

Fig. 2. Brood patterns for the 12 days following irradiation. ○, *XXY* females produced by non-disjunction in immature sperm; ●, males with B^s marker deleted; ▲, males with y^+ marker deleted

cross are wild-type (heterozygous) females and non-yellow, apricot, echinus, Bar males. Complete loss of X or Y chromosomes will yield sterile $X0$ males phenotypically yellow, apricot, echinus, non-Bar. Deletion of either y^+ or B^s marker from the Y chromosome will yield yellow, apricot, echinus, Bar males (abbreviated to yellow males) or non-yellow, apricot, echinus, non-Bar males (or simply non-Bar males) respectively. Non-disjunction of X and Y will lead to the recovery of Bar females with XXY constitution. Where possible, all exceptional flies were analysed genetically to confirm this classification.

Fig. 1 shows the comparative yields of $X0$ males and of non-disjunctional females while the frequencies of each deleted Y chromosome class and of non-disjunctional females are given in Fig. 2. On the assumption that the determination of non-disjunction will occur only at or before anaphase in the first meiotic division, then it seems likely that the seventh brood in particular represents treated pre-meiotic stages. These results also confirm the high sensitivity of meiotic and immediately pre-meiotic stages—presumably primary spermatocytes—to the mutagenic effect of X-rays.

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A Gene synthesizing Protease and a Pre-Viral Genome in Silkworms

Almost twenty years ago[1], we put forward a working hypothesis that viruses may originate in nucleoproteins in cells, and in 1943 [2] we discovered that the viral nuclear polyhedrosis of larvæ of *Bombyx mori* can be induced by a metabolite. The later development of work on induction has suggested[3] that the polyhedral virus would be derived from the chromosomal segment containing the genes controlling the synthesis of protease and deoxyribonuclease. We have now demonstrated that the pre-viral genome contains a gene synthesizing an alkaline protease and is located in the chromosome of healthy silkworms.

Our previous investigation[4] showed that the pupa of *B. mori* has no alkaline protease and never produces polyhedra without infection. About 200 pupæ of the Zelem × plem strain were each injected with 0·02 ml. of polyhedral solution (0·1 mgm./ml.), and polyhedra were collected in the cold by our method[5] from infected individuals before death. Viral particles were then isolated from the polyhedral crystals by the procedure previously described[6] and polyhedral protein from the crystals was also prepared at the same time. However, the first centrifugation was carried out immediately after dissolving polyhedra in a mixture of sodium carbonate and sodium chloride, without standing overnight. The proteolytic action of viral particles and polyhedral protein obtained was measured by the method previously reported[6]. The results are given in Table 1, in which the protease activity is expressed in μgm. tyrosine per hr. produced by 10 mgm. of the particles or protein.

Table 1. Proteolytic Action of Polyhedral Virus

pH of reaction mixture		8·2	9·0	9·8	10·5	11·0	11·5
μgm. of tyrosine	Viral particles	4	10	17	28	31	29
	Polyhedral protein	1	3	7	13	14	12

It is thus evident that the polyhedrosis virus forms a protease which shows the highest activity at pH 10·5–11·0. As the polyhedral virus is of deoxyribonucleic acid nature and produced only in the nucleus, it can be concluded that there exists in the virus a gene which possesses the ability to synthesize the alkaline protease.

Our previous experiments[5,7] have further shown that the *B. mori* larva has an alkaline protease and produces polyhedra containing a protease of the same nature. The white cuticle of the p_{44} strain and the black cuticle of the p^s strain respectively were taken in the midst of the final diapause, namely just at the end of the formation of new cuticle during the third ecdysis, and 0·1 gm. of the cuticle consisting of epi-, exo- and endo-cuticle was ground with 10 ml. of 0·02 N sodium carbonate.

These two strains were then crossed and the cuticle from the descendants in the F_1- and F_2-generations was treated with the sodium carbonate solution. The proteolytic action of the suspensions obtained was immediately measured at pH 10·5 in the same way as before[6]. The results are given in Table 2, in which the protease activity is expressed in μgm. tyrosine produced by 10 mgm. of cuticle per hr.

Table 2. Proteolytic Action of Larval Cuticle

Generation	p_{44}	p^s	$p_{44} \times p^s - F_1$	$p_{44} \times p^s - F_2$	
Colour of cuticle	White	Black	Black	White	Black
μgm. of tyrosine	81	161	183	71	181

It is thus obvious that there exists a correlation between the proteolytic activity and colour of the skin, one of the tissues in which polyhedral virus is produced. As it has already been established[8] that the plain white of the p_{44}-cuticle and the striped black of the p^s-cuticle are governed by the genes in the second chromosome in the larval cell, and that the latter is the dominant, we may conclude that the gene synthesizing the alkaline protease is located in the chromosomes, possibly in the second chromosome. Actually in an experiment the number of white and black larvæ in one group of the F_2-generation was 138 and 429, respectively. Similar results concerning the activity of cuticular protease and the ratio of F_2 larvæ were also obtained in the case of the reciprocal cross.

In view of these observations, it seems reasonable to assume that, by the inducing process, this special pre-viral genome in larval chromosomes can be activated to form the polyhedral virus.

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[4] Yamafuji, K., and Yoshihara, F., *Enzymologia*, **22**, 157 (1960).
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[7] Yoshihara, F., *Sci. Bull. Fac. Agric. Kyushu Univ.*, **18**, 139 (1960).
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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 27

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Prof. A. G. MacGregor: "Endocrine Interdependence".*

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. F. A. E. Pirani and Dr. G. F. Elliott: "Ballistic Missiles and Nuclear Weapons—The Development of Weapons Technology".* (Last of seven lectures in War Studies.)

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. D. Müller (Copenhagen): "Microbiological Formation of Hydrocyanic Acid".*

Tuesday, November 28

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. G. B. Brown: "What is Wrong with Relativity ?"*

ROYAL INSTITUTION OF NAVAL ARCHITECTS (at the Institute of Marine Engineers, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Dr. J. E. Richards: "High Temperature Gas-Cooled Reactors for Marine Propulsion".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. E. G. Gray: "Electron Microscopy of C.N.S.".* (Thirteenth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. R. Popper: "Science and Growth of Knowledge".* Third of four Shearman Memorial Lectures.)

UNIVERSITY OF LONDON (in the Eugenics Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. Pontecorvo, F.R.S.: "Genetic Analysis via Mitotic Segregation".*

UNIVERSITY OF LONDON (at the Institute of Archæology, 31–34 Gordon Square, London, W.C.1), at 5.45 p.m.—Mr. Emmanuel Anati: "The Rock Engravings of the Negev Desert, Israel".*

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. C. A. Finch: "Polyolefin Fibres and Films".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. R. F. Rodger: "Automatic Plant Analysis by Electrochemical Methods".

Wednesday, November 29

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. Wolfendale: "Recent Developments in Semiconductor Devices and their Applications".

UNIVERSITY OF LONDON (in the Eugenics Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. G. Pontecorvo, F.R.S.: "Prospects for Genetic Analysis in Man".*

BRITISH INSTITUTION OF RADIO ENGINEERS, ELECTRO-ACOUSTICS GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. R. Yorke: "An Experimental Assessment of Loudspeaker Performance".*

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. Paul Adorian: "Television in Schools".

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Mr. B. H. Venning: "The Response of Dynamic Systems to Shock Excitation".

INSTITUTE OF INFORMATION SCIENTISTS (at A.E.I. House, 33 Grosvenor Place, London, S.W.1), at 6.30 p.m.—Dr. A. R. Michaelis: "Films for the Communication of Science".

ROYAL INSTITUTE OF CHEMISTRY (at Shell-Mex House, Strand, London, W.C.2), at 6.30 p.m.—Annual General Meeting.

Wednesday, November 29—Thursday, November 30

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULIC PLANT AND MACHINERY GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 10.45 a.m. on Wednesday and 10.30 a.m. Thursday—Conference on "Oil Hydraulic Power Transmission and Control".

Wednesday, November 29—Friday, December 1

IRON AND STEEL INSTITUTE (at the Federation of British Industries, Tothill Street, London, S.W.1, and at the Iron and Steel Institute, 4 Grosvenor Gardens, London, S.W.1)—Autumn General Meeting, and Forty-third Meeting of the Iron and Steel Engineers Group.

Thursday, November 30

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Anniversary Meeting.

UNIVERSITY OF LONDON (at the Wright–Fleming Institute of Microbiology, St. Mary's Hospital Medical School, Norfolk Place, London, W.2), at 5 p.m.—Prof. R. Hare: "Sir Almroth Wright" (Commemorative Lecture).*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. I. Friedmann: "Electron Microscopy in Pathology of the Ear, Nose and Throat".* (Fourteenth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. R. Popper: "Science and the Growth of Knowledge".* (Last of four Shearman Memorial Lectures.)

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the BATTERSEA COLLEGE OF TECHNOLOGY CHEMICAL SOCIETY, at the Battersea College of Technology, Battersea Park Road, London, S.W.11), at 7 p.m.—Dr. C. C. Hall: "Chemical Aspects of the Work of the Warren Spring Laboratory".

Thursday, November 30—Friday, December 1

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (in conjunction with the BRITISH NUCLEAR ENERGY CONFERENCE, at Savoy Place, London, W.C.2)—Symposium on "Nuclear Electronics".

Friday, December 1

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Mr. E. B. Worthington: "The Science of Nature Conservation".

Saturday, December 2

ASSOCIATION OF AGRICULTURE, and the BRITISH AGRICULTURAL HISTORY SOCIETY (at the London School of Economics, Houghton Street, Aldwych, London, W.C.2), at 11 a.m.—Agricultural History Conference.

BRITISH MYCOLOGICAL SOCIETY (at the Linnean Society of London, Burlington House, Piccadilly, London, W.1), at 2 p.m.—Annual General Meeting, followed by Miss G. M. Waterhouse: "The Zoospore" (Presidential Address).

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. W. K. Ford: "Tropical Insect Life".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT (male, with a degree in chemistry and interests in biochemistry), to assist in a Medical Research Council supported programme of research into dental disease—The Secretary, London Hospital Medical College, Turner Street, London, E.1 (December 2).

ASSISTANT LECTURERS OR LECTURERS IN EXPERIMENTAL PSYCHOLOGY, APPLIED MATHEMATICS, PURE MATHEMATICS, INORGANIC CHEMISTRY, PHYSICAL CHEMISTRY, APPLIED MATHEMATICS OR STATISTICS, MECHANICAL ENGINEERING, CIVIL ENGINEERING, and PRODUCTION ENGINEERING—The Registrar, The University, Nottingham (December 9).

LECTURERS IN MATERIALS IN THE DEPARTMENT OF ENGINEERING, to be responsible for equipping a new materials laboratory and teaching modern physical theories of material behaviour—The Registrar, The University, Leicester (December 9).

LECTURER IN SOCIAL AND DEVELOPMENTAL PSYCHOLOGY—The Registrar, The University, Leicester (December 9).

SENIOR LECTURER IN THE DEPARTMENT OF BIOCHEMISTRY—The Registrar, The University, Leicester (December 9).

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree or equivalent qualification) IN THE DEPARTMENT OF PHYSIOLOGY AND PLANT CULTURE, to assist in physiological studies in controlled environments—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (December 10).

SENIOR LECTURER and a LECTURER IN THE DEPARTMENT OF MATHEMATICS, University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 20).

CHAIR OF APPLIED PHYSICS—The Registrar, The University, Hull (December 31).

CHAIR OF SOCIOLOGY—The Registrar, The University, Hull (December 31).

LECTURER OR SENIOR LECTURER IN GENETICS at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, December 31).

LECTURER IN BACTERIOLOGY IN THE SCHOOL OF VETERINARY MEDICINE—The Registrar, Trinity College, Dublin 2 (January 1).

CHAIR OF THEORETICAL AND INORGANIC CHEMISTRY in Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, January 10).

LECTURERS IN PHYSICS—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne 1 (January 15).

LECTURER OR ASSISTANT LECTURER IN ZOOLOGY at the University of Malaya, Singapore—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 15).

RESEARCH OFFICER (preferably with an honours degree in science, agriculture or forestry, and experience in research) OF THE TUSSOCK GRASSLANDS AND MOUNTAIN LANDS INSTITUTE—The Registrar, Lincoln College, Lincoln, New Zealand (January 15).

CHAIR OF ENGINEERING in the University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Common-

wealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, January 31).

Lecturers or Assistant Lecturers (3) (with a special interest in organic or inorganic or physical chemistry) in Chemistry at the University College of The West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 31).

Assistant Experimental Officer (with a pass degree or equivalent and experience in biological work), for work on the flow and coagulation of blood—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref. 61/19.

Assistant Lecturer, Grade B (with a good honours degree in mathematics and preferably some teaching or research experience) in Mathematics—Clerk to the Governors, South-East Essex Technical College, Longbridge Road, Dagenham, Essex.

Assistant Professor in the Department of Physics—Head, Department of Physics, Dalhousie University, Halifax, Canada.

Assistant Professor of Physics—Prof. C. E. Challice, Department of Physics, University of Alberta, Calgary, Alberta, Canada.

Biology Graduate (preferably with a knowledge of immunological technique) in the Endocrine Department, to do research on gonadotrophins—The Secretary, University College Hospital Medical School, University Street, London, W.C.1.

Laboratory Technician, for a research laboratory specializing in steroid biochemistry—Dr. Ludwig Kornel, Division of Endocrinology, University of Alabama Medical Center, Birmingham 3, Alabama, U.S.A.

Medical Assistant Editor (registered medical practitioner not older than 45 years) to the *British Medical Journal*—The Editor, *British Medical Journal*, B.M.A. House, Tavistock Square, London, W.C.1, marking envelope "*British Medical Journal*—Assistant Editor".

Postdoctoral Fellow in the Department of Biochemistry—C. S. Hanes, Department of Biochemistry, The University, Toronto.

Postgraduate Research Assistant (with an honours degree or equivalent) in Physical-Organic Chemistry—The Head, Chemistry Department, University of Saskatchewan, Saskatoon, Canada.

Research Assistant, for work on the clathration and occlusion of rare gases—Prof. R. M. Barrer, Chemistry Department, Imperial College of Science and Technology, Loadon, S.W.7.

Research Assistant in the Department of Pathology, for tissue culture work on the nervous system—The Professor of Pathology, School of Medicine, The University, Leeds 2.

Research Assistant (with a degree or H.N.C. in electrical engineering or applied physics, or an equivalent qualification, and preferably some experience in electronics and/or vacuum techniques), for work on the High Voltage Electron Microscopic project—The Secretary, Department of Physics, Cavendish Laboratory, Free School Lane, Cambridge.

Senior Lecturer (graduate of a British university) in Biochemistry—Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

Technician in Biochemistry (A.I.M.L.T., B.Sc., or A.R.I.C.)—The Director, Department of Pathology, Paddington General Hospital, London, W.9.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 136: Facilities for Agricultural Education. Pp. 8. (Belfast: Ministry of Agriculture, 1961.) [19

Imperial College of Science and Technology. Shorter Postgraduate Courses, Session 1961–62, Autumn Term. Pp. 20. (London: Imperial College of Science and Technology, 1961.) [19

The Institution of Electrical Engineers. Regulations for the Electrical Equipment of Ships. Fourth edition. Pp. v+125. (London: The Institution of Electrical Engineers, 1961.) Limp cloth, 7*s.* 6*d.*; Case-bound in cloth, 10*s.* [19

Freshwater Biological Association. Scientific Publication No. 20: A Key to the Nymphs of the British Species of Ephemeroptera, with Notes on their Ecology. By Dr. T. T. Macan. Pp. 64. (Ambleside: Freshwater Biological Association, 1961.) 4*s.* 6*d.* [19

Berkshire, Buckinghamshire and Oxfordshire Naturalists' Trust, Ltd. First Annual Report, 1960. Pp. 7. (Kennington, Oxford: Berkshire, Buckinghamshire and Oxfordshire Trust, Ltd., 146a Poplar Grove, 1961.) [19

Department of Scientific and Industrial Research. Summary of Progress of the Geological Survey of Great Britain and the Museum of Practical Geology for the year 1960, with Report of the Geological Survey Board. Pp. iii+78. (London: H.M. Stationery Office, 1961.) 5*s.* net. [19

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 708, Vol. 244 (31 August, 1961): The Anatomy of the Nervous System in the Genus *Gerris* (Hemiptera-Heteroptera). By D. M. Guthrie. Pp. 65–102+plate 8. 14*s.* No. 709, Vol. 244 (31 August, 1961): Triassic Reptiles from the Elgin Area: *Stagonolepis*, *Dasygnathus* and their Allies. By A. D. Walker. Pp. 103–204+plates 9–13. 40*s.* (London: Royal Society, 1961.) [19

Department of Agriculture and Fisheries for Scotland. Marine Research No. 3 (1961): Scottish Oyster Investigations, 1946–1958. By Dr. R. H. Millar. Pp. 76. (Edinburgh and London: H.M. Stationery Office, 1961.) 22*s.* 6*d.* net. [59

The Wild Resources of East and Central Africa: a Report following a visit to Kenya, Uganda, Tanganyika, Northern and Southern Rhodesia, and Nyasaland, in February and March, 1960. By Dr. E. B. Worthington. Pp. ii+26. (Colonial No. 352.) (London: H.M. Stationery Office, 1961.) 2*s.* 6*d.* net. [59

Royal Observatory Bulletins. No. 38: Double-Star Observations made with the 28-inch Refractor 1957–1960. By R. v. d. R. Woolley, D. H. P. Jones and M. P. Candy. Pp. i+E207–E241. (London: H.M. Stationery Office, 1961.) 5*s.* 6*d.* net. [59

Other Countries

Uganda Protectorate. Report of the Forest Department for the period 1st January, 1959 to 30th June, 1960. Pp. ix+80. (Entebbe: Government Printer, 1961.) Shs. 5/20. [19

Tea Research Institute of East Africa. Annual Report for the year 1960. Pp. 50. (Kericho, Kenya Colony: Tea Research Institute of East Africa, 1961.) [19

Sandia Corporation Monographs. SCR–409: Output Mean and Deviation Versus Input S/N for Linear Quadratic, and Quantizing Envelope Detectors with Saturation. By Charles S. Williams, Jr. Pp. 38. (Albuquerque, New Mexico: Sandia Corporation, 1961. Obtainable from Office of Technical Services, Department of Commerce, Washington, D.C.). 1 dollar. [19

Camera di Commercio Industria e Agricoltura di Milano. *La Stampa Periodica Italiana*, Nos. 1–2, 1961. Pp. ii+32. (Milano: Camera di Commercio Industria e Agricoltura, 1961.) [19

Forschungsberichte des Landes Nordrhein-Westfalen. Nr. 919: Die Haarfarben der Säugetiere. 1: Spektralanalytische Untersuchungen an Haut- Haar- und Federmelaninen (Literaturübericht). Von Dr. Ernst Lubnow und Lisa Kramer. Pp. 74. (Köln und Opladen: Westdeutscher Verlag, 1961.) 2,30 D.M. [19

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 61–8: Triassic Stratigraphy of the Rocky Mountains and Foothills, Northeastern British Columbia, 94 K and N (parts of). By B. R. Pelletier. Pp. iv+32. (Ottawa: Queen's Printer, 1961.) 50 cents. [59

New Zealand: Department of Education. Post-Primary School Bulletin, Vol. 14, No. 3: Writing in New Zealand—The Scientists. By G. A. Elby. Pp. 32. (Wellington: Government Printer, 1961.) [59

A General Report of the Makerere Expedition to Lake Manyara, April–July, 1961. Pp. i+22+map. (Kampala, Uganda: Makerere College, University College of East Africa, 1961.) [59

Republic of South Africa. Department of Commerce and Industries: Division of Fisheries. Thirteenth Annual Report for the period 1st April, 1958 to 31st March, 1959. (Reprinted from *Commerce and Industry*, March, 1961.) Pp. 160. (Pretoria: Government Printer, 1961.) [59

International Scientific Radio Union. Proceedings of the XIIIth General Assembly held in London, September 5–15, 1960. Vol. XII, Part 2: Commission II of Radio and Troposphere. Pp. 143. (Brussels: International Scientific Radio Union, 1961.) [59

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Map 12–1961: Sheldon Lake, Yukon Territory. Map 13–1961: Tay River, Yukon Territory. Map 29–1961: Surficial Geology—Montreal Area, Quebec. (Ottawa: Geological Survey of Canada, 1961.) [59

Institut des Parcs Nationaux du Congo et du Ruanda-Urundi. Beobachtungen an Giraffen in Zoologischen Gärten und Freier Wildbahn. Von D. Backhaus. Pp. 202. Exploration du Parc National Albert (Deuxième Série). Mission Henri H. Mollaret. Fascicule 11: Biotopes de Haute Altitude Ruwenzori (II) et Virunga—Etudes Diverses. Pp. 101+3 planches. (Bruxelles: Institut des Parcs Nationaux du Congo et du Ruanda-Urundi, 1961.) [59

Institut des Parcs Nationaux du Congo et du Ruanda-Urundi. Exploration du Parc National de la Garamba. Mission H. de Saeger en collaboration avec P. Baert, G. Demoulin, I. Denisoff, J. Martin, M. Micha, A. Noirfalise, P. Schoemaker, G. Troupin et J. Verschurem (1949–1952). Fascicule 20. (1): Meenoplidae. Par H. Synave. (2) Fulgoridae. Par H. Synave. (3): Sphecidae. Par Jean Leclercq. Pp. 105. Fascicule 21. (1): Cestodes. Par Jean G. Baer et Alexandre Fain. (2): Euaesthetinae. By David H. Kistnet. (3) Lamiinae. Par Stephan Breuning. (4): Onthophagini. Von Georg Frey. (5): Tabanidae. Par Marcel Leclercq. Pp. 115. Fascicule 23: Toxopodinae, Sepsidae (Diptera Tetanoceridea). Par Paul Vanschuytbroeck. Pp. 80. Exploration du Parc National de l'Upemba. Mission G. F. de Witte en collaboration avec W. Adam, A. Lanssens, L. Van Meel et R. Verheyen (1946–1949). Fascicule 60. (1): Genre *Heliocopris* (Coleoptera Polyphaga Fam. Scarabaeidae). Par André Janssens. (2): *Athalia* Leach (Hymenoptera Tenthredinidae). By Robert B. Benson. (3): *Pseudini* (Hymenoptera Sphecoidea Pemphredoninae). Par Jean Leclercq. (4): Proctotrupidae, Key to the genera of the World. Pp. 47. Fascicule 61: *Scio-myzidae* (Diptera Brachycera Malacophaga). Par Jean Verbeke. Pp. 37. Fascicule 64: The Food of Amphibians. By Robert Inger and Hymen Marx. Pp. 86. (Bruxelles: Institut des Parcs Nationaux du Congo et du Ruanda-Urundi, 1961.) [59

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Geological Maps. Index Sheet 82: British Columbia—Alberta. Index Sheet 83: Alberta—British Columbia. Index Sheet 93: British Columbia. Index Sheet 94: British Columbia. Index Sheets 104 and 114E: British Columbia. (Ottawa: Geological Survey of Canada, 1960.) [59

Deep-Water Eels from Cook Strait, New Zealand. By P. H. J. Castle. (Zoology publications from Victoria University of Wellington, No. 27.) Pp. 30. (Wellington: Victoria University of Wellington, 1961.) [79

New Zealand. Report of the Department of Scientific and Industrial Research for the year ended 31 March 1961. (H. 34.) Pp. 87. (Wellington: Government Printer, 1961.) [79

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